AD

RESEARCH AND DEVELOPMENT ON INHALATION TOXICOLOGIC EVALUATION OF RED PHOSPHORUS/BUTYL RUBBER COMBUSTION PRODUCTS

PHASE II REPORT

CATHERINE ARANYI

DECEMBER 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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IIT Research Institute, Life Sciences Research Division
10 West 35th Street, Chicago, Illinois 60616

Contracting Officer's Technical Representative

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Exploratory ale and female Sprague-Dawley rats exposed to concentrations ranging from 0.5 to 4 mg/l for 1 to 3 hr periods showed highl creases in pulmonary bactericidal activity to inhaled 35S-K enerally significant decreases in total cell counts in the pulmc obtained by trachebronchial lavage. Subsequent range findir quested that exposure concentration is the determining facto atter than duration and demonstrated that LC50 (continued)

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studies with single exposures, as required in the contract, were not feasible in the range of aerosol concentrations physically attainable with the generators at the air flow rates required in our inhalation exposure system. When, in a follow-up multiple exposure study, rats innaled RP/BR aerosols for 1 hr/day for 5 days at concentrations from 1.56 to 3.05 mg/l mortality ranged from 5 to 90 percent and the estimated LC50 value was 2.32 mg/l. When these data were contrasted with observations from two studies with 5 daily 4-hr exposures to 0.35 or 0.99 mg/l of RP/BR the importance of exposure concentration over duration in terms of producing more pronounced effects at similar CxT values was evident again. Results of the definitive range finding studies designed on the basis of these exploratory experiments demonstrated that inhalation of 0.5 mg/l RP/BR aerosol for 1 or 3.5 hr daily for 4 days, or for 3.5 hr 4 times weekly for 4 weeks produced decreases in body weights relative to control animals, but the exposures did not result in gross pathologic changes and the microscopic changes observed in lung tissue could not be considered compound-related. The changes found in pulmonary lavage parameters after 4 exposures were no longer present after the recovery period.

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EXECUTIVE SUMMARY

The objective of these studies is to develop a data base for health hazard assessment of the effects produced by inhalation of combustion products from red phosphorus/butyl rubber used as an obscurant smoke for troops and vehicles in tactical and training environments. Laboratory rats exposed in inhalation chambers are used to provide a comprehensive definition of the biologic effects of red phosphorus smoke to mammalian systems under conditions which approximate the potential troop exposure. This report summarizes the Phase II range finding studies.

In early exploratory experiments male and female Sprague-Dawley rats were exposed to various concentrations of RP/BR aerosols ranging from 0.5 to 3 mg/l for durations of 1- to 4-hr. Highly significant decreases in pulmonary bactericidal activity to inhaled ^{35}S-*K.pneumoniae* were found in the exposed rats relative to controls after single as well as multiple exposures. The higher exposure concentrations also produced significant decreases in total cell counts in the pulmonary free cells obtained by tracheobronchial lavage from the exposed rats relative to those collected from controls.

In subsequent range finding mortality studies male and female rats were given single 1-hr exposures to 2.00, 2.22, 2.62, 3.09 and 3.15 mg/l of RP/BR aerosols and observed for 14 days. Only the exposures to $\geq$2.62 mg/l caused deaths. The maximum mortality resulting from a single 1-hr exposure to approximately 3 mg/l of RP/BR aerosol (maximum generator capacity) was 20 to 25 percent, while 2.62 mg/l resulted in 6 percent deaths. A single 4-hr exposure to 0.88 mg/l with a CxT value similar to those in the 3.09 and 3.15 mg/l 1-hr studies caused no deaths thereby suggesting that exposure concentration is the determining factor rather than duration. These experiments demonstrated that LC studies with single exposures at logarithmically spaced concentrations, were not feasible in the range of aerosol concentrations levels physically attainable with the generators at the air flow rates required in our inhalation exposure system.

In a follow-up multiple exposure study rats inhaled RP/BR aerosols for 1 hr daily on 5 consecutive days at concentrations of 1.56, 1.99, 2.49 and 3.05 mg/l. Mortality rates, mean survival times, body weights and overall clinical observations were made during the 5 exposure days and for 14-day observation period. Necropsies were done on all animals that died during the study and on survivors on Day 19. Mortality ranged from 5 to 90 percent decreases in survival times. The estimated LC : value was 2.32 mg/l with 1.99 and 2.73 mg/l confidence limits.

When rats received five daily 4-hr exposures to 0.35 or 0.99 mg/l of RP/BR aerosol only one died. Comparison of body weights and of clinical observations for the 1- and 4-hr studies showed markedly

adverse effects after the 1-hr and practically negligible ones after the 4-hr exposures. Thus the greater influence of exposure concentration on toxicity over duration was again demonstrated.

Based on the results of these range finding experiments the definitive studies were conducted on male and female rats using four daily exposures to 0.5 mg/l of RP/BR aerosol and comparing 1.0 and 3.5-hr exposure durations. Pulmonary free cell parameters and structural changes, as evaluated by gross necropsy and histopathology, were tested immediately following the last exposure and after a 14-day recovery period. In an additional study rats were exposed to 0.5 mg/l of RP/BR for 3.5-hr daily 4 days per week for 4 weeks. Gross pathologic observations were made after the last exposure and 2 weeks following the last exposure. All studies included daily clinical observations and regular body weight determinations.

No deaths occurred in any of the studies and generally no treatment-related clinical observations were seen. Statistical analysis of body weights showed that exposure duration was not a significant factor. When body weight changes were analyzed over the entire period of exposure and recovery, the 4-day as well as 4- week exposures produced weight decreases in male and female rats relative to controls that did not return to the normal control level within the recovery period.

Exposure duration did not have a significant effect on the pulmonary free cell parameters. Total and differential cell counts in the pulmonary lavage were not affected. Cellular ATP levels in the lavaged cells were significantly decreased when examined immediately after the fourth exposure, however recovery was complete after 14 days.

No compound-related gross pathologic lesions were observed during necropsies immediately following the last exposure or after a 14-day recovery period in either the 4-day or the 4-week exposure group.

Microscopic examination of the tissues immediately following the 4-day exposure and after a 2-week recovery period did not reveal any treatment- related changes in kidneys, trachea and nasal turbinates. However, lungs of several animals had focal areas of interstitial reaction with alveolar macrophages which may be treatment-related. These animals, however, also had moderate to marked lymphoid hyperplasia of pulmonary lymph nodes which suggests an infectious agent may have produced the pulmonary lesions.

FOREWORD

This report, IITRI No. L06139, Phase II Report describes studies conducted by the Life Sciences Division, IIT Research Institute for the Health Effects Research Division, U.S. Army Medical Bioengineering Research and Development Laboratory during the period of February through July 1983. The studies were carried out under Contract No. DAMD17-82-C-2121.

Catherine Aranyi served as Principal Investigator and James Fenters was Co-Investigator. Principal professional associates were Stanley Vana and Jeannie Bradof. Mr. Vana was responsible for the inhalation exposure facilities and the aerosol generation and monitoring throughout the studies. Ms. Bradof was in charge of all pulmonary response studies and overall toxicologic observations. Necropsy procedures, tissue collection and preparation for histopathologic evaluation were under the supervision of Vladislava Rac and Carol Thompson. Percent phosphoric acid analysis in the filter-collected aerosol samples was performed under the supervision of Alan Snelson. Robert Gibbons, Consultant Biostatistician performed statistical analysis of the experimental data. Histopathologic evaluation of the collected tissue samples was performed by W. D. Iverson, consultant pathologist from Experimental Pathology Laboratories, Herndon, VA.

Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1978), DHEW Publication No. (NIH) 78-23 prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council; regulations and standards of the Department of Agriculture and Public Law 91-579, "Laboratory Animal Welfare Act" (1970).

Citation of commercial organizations and trade names in this report does not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

QUALITY ASSURANCE STATEMENT

Laboratory operations were inspected on May 16, 18, 19 and 24, June 3 and September 28, 1983. Final draft reports were audited on August 8 and December 9 to 14, 1983. Inspection and audits were performed by Josephine M. Reed, Julie McPhillips and Kirit Parikh.

The study was found to conform to IITRI Life Sciences Quality Assurance criteria developed to meet FDA Good Laboratory Practice Regulations (Fed. Reg. CFR, Part 58, 1978). Animal experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" (1978), DHEW Publication No. (NIH) 78-23 prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council; regulations and standards of the Department of Agriculture and Public Law 91-579, "Laboratory Animal Welfare Act" (1970).

Raw data generated during the course of the study will be retained in the IITRI Life Sciences Archives.

Josephine M. Reed
Supervisor, Quality Assurance

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I. INTRODUCTION

As part of an overall concern for personnel health and safety, the U.S. Army Medical Research and Development Command is seeking to evaluate the effects produced by inhalation of combustion products from red phosphorus/butyl rubber used as an obscurant smoke for troops and vehicles in tactical and training environments. Laboratory rats, exposed in chambers will be used to provide a comprehensive definition of the biologic effects of red phosphorus smoke to mammalian systems under conditions which approximate the potential troop exposure. The approach to this research includes range finding acute studies to determine lethal concentrations and influence of exposure duration on mortality; repeated exposure studies to define time-concentration relationships as well as threshold levels, healing, and adaptation in biologic reactions; and a subchronic exposure study with a recovery and observation period after the experimental exposure. The principal biologic response criteria to be monitored include overt toxic signs, clinical chemistry and hematology, histopathology, alveolar macrophage defense functions, pulmonary bactericidal activity and neurobehavioral activity. The research project is set up to proceed in a phased manner. The Phase II biologic range finding studies summarized in this report were conducted to form the basis for the selection of exposure concentration levels, durations and frequencies to be used in the Phase III intermediate-term multiple exposures.

II. MATERIALS AND METHODS

A. Animals

Male and female Sprague Dawley rats, 3- to 4-weeks-old were obtained from Harlan/Sprague-Dawley, Inc., Madison, WI. The rats were housed 3 per cage during the quarantine period in plastic cages with water bottles. Following test animal selection the rats were housed individually in stainless steel inhalation cages containing four compartments, each compartment measuring 18.4 x 16.5 x 15.9 cm. The cages were used for holding as well as exposure throughout the studies. When attached to the racks the cages are equipped with an automatic drinking water distribution system and are suspended over excrement pans. For exposures the cages are removed from the racks. The animals were transferred weekly to clean cages and the deotized absorbing cage boards were changed twice per week.

The animal rooms were maintained on 12-hr light/dark cycle. Temperature and relative humidity were regulated in such a manner that extreme fluctuations between the animal room and exposure

chamber conditions would be avoided. Purina Certified Rodent Chow 5002 and water were available to the rats ad libitum except during the exposures.

Animals were randomized by sex to treatment groups using a constrained random process, stratified by weight, such that all groups tested were comparable in pretest body weight, but assignment of individual animal to groups was random. Each test animal was identified with a unique number attached through ear tags.

B. Generation and Monitoring of the Chamber Atmosphere

1. Red Phosphorus/Butyl Rubber (RP/BR)

The test article, RP/BR softened with hexane and prepackaged in 0.75 in diameter 4.5 in long stainless steel feed cylinders (billets) with end caps was supplied by the Sponsor through Oak Ridge National Laboratories (ORNL) and was stored at ambient temperature. A record of the test article was maintained which included date of receipt with identification numbers of each cylinder and the date and study number for which it was used.

2. Inhalation Exposure Facility

The inhalation exposure facilities and methods have been described in detail in the Phase I Report (1). Its major components are the inhalation exposure chambers with air flow and differential pressure controls; the conditioned air supply and chamber air exhaust systems; the red phosphorus/butyl rubber combustion generators and the aerosol monitoring systems.

Supply air to the inhalation exposure laboratory was preconditioned by passing through prefilters, charcoal filters and an air conditioning unit. Automatically controlled heating and humidifying units built into the supply air system maintained the air temperature and relative humidity (RH) at the specified ranges of 24 to 27°C and 40 to 60 percent RH. Prior to entering the exposure chambers the conditioned air was further filtered through a fiberglass coarse filter, a charcoal bed and HEPA filter.

The combined exhaust air from the exposure chambers was filtered through a single-housing, 30-element coalescent filter and exhausted above the roof of the building. The control chamber exhaust was independent from that of the experimental test chambers to avoid potential contamination from the test aerosol. The negative pressure and airflow rate in the chambers and exhaust filter loading were monitored by differential pressure gauges.

The aerosol was generated by specially designed hydraulic extrusion-combustion generators provided by the Government through

ORNL. The generator operates by exerting hydraulic pressure on the RP/BR forcing it to extrude from an orifice into a burn chamber where it is ignited. The aerosol generated from the combustion products is transported directly into the exposure chamber inlet port. At a constant chamber airflow rate the concentration of the aerosol is a function of the extrusion rate of the RP/BR which is controlled by a precision hydraulic metering pump.

The rats were exposed to the test atmosphere in identical 1-m^3 sized stainless steel inhalation chambers operating at a total airflow rate of 500 L/min. Aerosol exposure and filtered air control chambers were located in separate laboratories.

3. Aerosol Monitoring

The RP/BR aerosol was monitored within each exposure chamber for mass concentration by gravimetric filter collection three times during the 1-hr exposures and four times during 3.5-hr exposure durations. In addition, mass concentration was monitored and output continuously recorded with light scattering photosensors. An integrated average of the photosensors was recorded simultaneously with the gravimetric filter sample collection. Aerosol particle size was determined with a Quartz Crystal Microbalance cascade impactor. The particle size was monitored in each chamber once on each exposure day. Determination of total phosphorus was conducted by spectrophotometric analys's of the collected filter samples on one filter per chamber per exposure week. All collected filters were recorded and stored in sealed containers until submitted for chemical analysis. Oxygen levels in the chambers were spot checked during each experiment and were consistently 21 percent.

C. Biological Endpoints

1. Pulmonary Bactericidal Activity to $[^{35}S]$-K. pneumoniae

Aerosols of $[^{35}S]$*Klebsiella pneumoniae* disseminated with a Retec X-70 disposable nebulizer were used for the bactericidal activity assay, using a method previously described for mice and adapted for rats (2). Radiolabeled *K. pneumoniae* were grown in a medium in which the sulfate requirement of the bacteria was provided by $[^{35}S]$sodium sulfate. Before aerosolization, the bacteria were washed repeatedly and centrifuged for removal of unattached radiolabel. Bacterial counts were determined in a Petroff-Hausser counting chamber by dark-field microscopy and by the culture plate technique. Radioactive counts were measured in a Mark III Liquid Scintillation System (Tracor Inc.).

The ra erial aerosol exposure chamber installed
In a glove of an 87-liter main compartment (adequate
for exposure hat was accessible through appropriate
airlocks thr e animals were moved. An additional small
airlock prov to the glove box for the nebulizers and
impingers. radioactive and pathogenic exposure hazard
to laborator all air exhausted from the chamber was
passed first n absolute filter placed within the glove box
and subseque gh a HEPA filter located on the outside.

Pulmona icidal activity was determined in the lungs of
the indivic 's from both exposed and control groups that
simultaneous aerosols of the viable radiolabeled
bacteria. the viable bacterial counts to the
radioactive h animal's lungs provided the rate at
which bacte ved 3 hr after infection. Thus, where

$$\text{ericidal Activity} = \frac{1-R_3}{K_o}\ 100$$

R^3 is o of bacterial to radioactive counts in the
lungs of i ual rats at 3 hr, and K0 is an average determined
from the sam os in the lungs of rats killed immediately after
inhaling th teria. This assay was used only in preliminary
studies.

2. Pulmonary Free Cells

Within 4 hr (0 hr) or 14 days after the last exposure, alveolar macrophages (AM) were obtained from designated animals by tracheobronchial lavage. The rats were weighed, killed with an overdose of sodium pentobarbital by intraperitoneal injection, and the lungs were lavaged through a blunted 18-gauge needle inserted into an incision in the trachea with 9 consecutive 6-ml infusions of warm saline. The AM were collected from the lavage fluids by centrifugation and resuspended in Hanks' balanced salt solution (HBSS) after the lavage fluids were collected for protein determination. Total cell counts were made in a hemocytometer. For determination of the cellular distribution (i.e., percent of AM, polymorphonuclear leukocytes and lymphocytes), differential counts were made on cytocentrifuge preparations of cells fixed in methanol and stained with Wright's stain.

Cellular adenosine triphosphate (ATP) levels were determined using a DuPont 760 Luminescence Biometer with the procedure recommended for the instrument. The assay is based on the principle that when a microsample containing ATP is injected into a suitably buffered reaction mixture of luciferase and luciferin, the peak intensity of the resulting light flash is directly proportional to the concentration of ATP. ATP was extracted from the cells by dimethyl sulfoxide (DMSO) from aliquots of the cell suspension. The DMSO extracts were diluted with a specially prepared 0.01 M morpholinopropane sulfonic acid (MOPS) buffer to overcome the quench effect of the high concentration of DMSO in the aqueous extract for the luciferase-luciferin reaction.

For determination of total cellular protein content, aliquots of the cell suspensions were treated with 1 percent sodium deoxycholate (SDC) and assayed by the Lowry method (3). Lavage fluids were assayed without SDC treatment. Total cellular protein values were given per 10^5 AM. ATP levels were expressed in femtograms (fg) of ATP per ug of total protein (experimentally determined), or per 10^5 AM (calculated from initial cell counts).

3. Standard Toxicology

Mortality was recorded daily and expressed as the percent of animals dying out of the total number of animals on test.

Mean survival time was estimated by the method of Horsfall (4), from the following equation:

$$MST = \frac{\Sigma(AxB) + (dxL)}{n}$$

where, A was the last day on which any individual rat was alive, B was the number of rats surviving A days, d was the last day of observation, L was the number of rats alive on day d, and n was the number in the experimental group.

Clinical observations: All animals were observed daily for survival, physical appearance, behavior and any pharmacologic and/or toxicologic signs. All observations were recorded on an individual test animal basis.

Body weight: Each animal was weighed using a Mettler PE 1600 balance with a special animal weighing mode at the initiation of the study and on exposure days daily before exposure. Initial preexposure weights were recorded as Study Day 1. Subsequent study days were numbered consecutively through the recovery period.

Necropsy and Histopathology: Rats were killed with carbon dioxide either within four hours or 14 days after the last exposure, bled from the dorsal aorta, and necropsied under the supervision of the pathologist. The necropsy procedure included a thorough, systemic examination and dissection of the animal viscera and carcass and collection and fixation of all major tissues.

All tissues and/or organs were examined *in situ* before dissection from the carcass for individual examination. Tongue, trachea, lungs and pulmonary lymph nodes were removed intact. Following weighing the lungs were infused via the endotracheal route with 10 percent neutral buffered formalin (NBF) before immersion in NBF. The head was removed and NBF was infused into nasal passages prior to submersion in NBF. All tissues were fixed not less than 24 hours prior to trimming.

Tissue trimming (wet sectioning) was performed at IITRI under the supervision and direction of a veterinary

pathologist. Organs were trimmed to allow the largest surface area possible for examination. Each lung lobe was sectioned along its main bronchus. Both a cross section and a longitudinal section of trachea was made.

The following tissues and/or organs were microscopically examined: The entire trachea, pulmonary lymph nodes, each lobe of the lungs, and two transverse sections of the skull through the nasal turbinates. (The first section was at the incisor's level. The second section was taken at the palatal ridge level). Since gross examination of the kidneys revealed mottling, these organs were also subjected to histological examination.

Tissues in paraffin blocks were shipped to Experimental Pathology Laboratories Inc. (EPL), Herndon, Virginia where hematoxylin and eosin stained slides were prepared and examined.

D. Statistical Methods

The LC_{50} concentration was calculated according to the method of Miller and Tainter by linear regression analysis (5). Comparisons made on the preliminary pulmonary lavage and bactericidal activity data were done using Student's t tests.

For Study Nos. 76/77 a four-factor mixed-model ANOVA was used to assess the effects of sex, exposure duration, treatment and replication on lavage parameters (cell count, cellular protein and ATP, and ATP per protein). A multivariate ANOVA for repeated measurements (growth curve model) was used to determine the effects of sex, exposure duration and treatment on repeated body weight measurements over time in Study Nos. 76/77 and 78. These models used in conjunction with this experimental design allow us to separate the effects of sex, exposure duration and treatment. In light of this, overall differences between males and females in body weight, for example, do not confound our estimation of treatment or duration effects. Furthermore, we also evaluated higher order interactions so that we may determine if, for example, a treatment effect is restricted to one sex group (i.e. sex by treatment interactions).

For the purpose of interpretation, individual post-hoc comparisons were performed only when a significant main effect or interaction was found. This allowed us to control the false positive rate and not be subject to problems inherent in multiple comparisons. In terms of sufficient statistics, means and standard deviations for each experimental condition were provided. In addition, combined mean values were provided for margins that were not statistically significant. For example, if exposure duration

was not a significant effect (i.e. no duration by treatment interaction), combined means averaging over duration conditions were provided. Combined standard deviations were not provided because they are not particularly relevant and may be misleading.

III. RESULTS

A. Preliminary Studies

In order to obtain preliminary information on the type and magnitude of the biologic responses produced by inhalation of RP/BR aerosols rats were placed into the exposure chambers during some of the exploratory aerosol generation studies of Phase I. These animals were examined for the effects of the exposures on *In vivo* pulmonary bactericidal activity and for changes in the pulmonary free cells obtained from their lungs by tracheobronchial lavage. Since the results of these experiments served as guidelines in the planning of the exposure conditions for the Phase II studies they have been included into this, instead of the Phase I report. Some of the rats used in these first exploratory experiments were kept in quarantine for evaluation of the automatic drinking water supply in the newly purchased cages and were older than usual. Subsequently 6-to 8-week-old rats were used in the remaining studies as required.

1. Pulmonary Bactericidal Activity

Immediately following exposure to RP/BR aerosol the experimental and control rats were simultaneously challenged with an aerosol of ^{35}S-*K. pneumoniae*. Pulmonary bactericidal activity determined 3 hr after inhalation of the bacteria was significantly depressed in the exposed compared to the control group in all but one of the exploratory experiments (Table 1). Highly significant decreases in bactericidal activity were found in lungs of 12- to 20-week-old male and female rats exposed to 1.88 mg/l of RP/BR aerosol for 1.5 hr, as well as in the lungs of 5-week-old male rats exposed to 1.08 mg/l RP/BR for 2.3 hr. The results after inhalation of approximately 0.5 mg/l of RP/BR in 6-week-old rats varied. Female rats exposed for 2.8 hr to 0.52 mg/l showed significant reduction in bactericidal activity. However when male and female rats were exposed to RP/BR at 0.63 mg/l for 3.0-hr pulmonary bactericidal activity, although reduced in both sexes, was significantly depressed in male rats only. In another exploratory experiment 7-week-old female rats were exposed 1.0 hr/day for 4 days to RP/BR aerosol at 0.54 mg/l. After 3 days rest, they were exposed for 1.0 hr/day for 3 additional days followed by aerosol challenge with ^{35}S-*K. pneumoniae* after the last exposure. Pulmonary bactericidal activity was significantly decreased in the exposed rats. Thus, repeated 1-hr inhalation exposures to approximately 0.5 mg/l of RP/BR aerosol, even when

Table 1. EFFECT OF EXPOSURE TO RP/BR AEROSOL ON THE PULMONARY BACTERICIDAL ACTIVITY OF RATS

Experiment No.	RP/BR Aerosol Exposure: Conc, mg/l[a] Mean	± SD	No. of Exposures	Duration hr per day	CXT	Rats: Sex	Age weeks	% of Inhaled *K. pneumoniae* Killed in 3 hr: Mean	± SE	n
1 a,b[b]	0							86.8	2.6	7
	1.88	0.05	1	1.5	2.8	M,F	12-20	46.5***	9.3	7
3 a,b[b]	0							88.5	2.1	9
	1.08	0.05	1	2.3	2.5	M	5	15.4***	7.5	10
9 a	0							64.0	4.4	8
	0.52	0.01	1	2.8	1.5	F	6	47.6*	4.8	11
23	0							70.5	5.2	8
	0.63	0.06	1	3.0	1.9	F	6	64.5	6.5	9
	0							80.2	3.5	7
	0.63	0.06	1	3.0	1.9	M	6	59.3*	6.2	11
75	0							80.4	1.9	13
	0.54	0.08	7[c]	1.0	3.8	F	7	73.3*	2.7	8

[a] Determined gravimetrically from multiple filter-collected aerosol samples.
[b] There was a 30 and 42 min interval between phases a and b of Experiments 1 and 3 respectively to recharge the generator with RP/BR.
[c] Four exposures, 3 days rest, and 3 exposures.
Difference from control: * $p < 0.05$
*** $p < 0.001$

interrupted with recovery days, resulted in significantly depressed pulmonary bactericidal activity.

2. Pulmonary Free Cells

In other exploratory experiments 11-to 13-week old male and/or female rats were exposed one time for 1 or 4 hr to RP/BR aerosols at 0.88, 2.22, or 3.18 mg/l, or for 4 daily 1-hr periods to 0.55 mg/l. Within 2 hr after the exposure the animals were killed with sodium pentobarbital, and pulmonary free cells were collected by *in situ* tracheobronchial lavage. Total and differential cell counts were made and total cellular protein levels were determined. The results summarized in Table 2 show consistently decreased total free cell yields and generally increased cellular protein levels in all groups of RP/BR-exposed animals compared to controls exposed to filtered air. The changes were statistically significant at the higher aerosol concentrations with 1 hr exposure durations (2.22 and 3.18 mg/l) and also in male rats exposed 4 times to daily 1 hr concentrations of 0.55 mg/l. Differential counts demonstrated that 96 to 100 percent of the cells were macrophages with occasional lymphocytes (not shown in the Table) present. No significant effects of RP/BR inhalation could be detected in cellular distribution.

B. Range-Finding Experiments

Range-finding experiments were conducted to provide the basis for the choice of the exposure concentrations to be tested in the definitive inhalation studies of Phase II. Mortality, survival time, body weights and in some experiments clinical observations were used to monitor the effects of inhalation of the RP/BR aerosols.

1. Single Exposures

In the first series of experiments male and female rats ranging in age from 6 to 13 weeks were given single exposures for approximately 1 hr to RP/BR aerosols ranging in concentration from 2.00 to 3.15 mg/l and mortality, survival time and body weight data were recorded over a 14-day period. As shown in Table 3, only exposure to 3.09 mg/l for over 1 hr caused deaths in male rats. Female rats died after single exposures to 2.62, 3.09 and 3.15 mg/l RP/BR. Mortality increased from 11 percent to 40 percent while mean survival time decreased from a 14.1 to 10.4 days with increasing RP/BR aerosol concentration. The maximum mortality resulting from a single 1-hr exposure to approximately 3 mg/l of RP/BR aerosol (maximum generator capacity) was 30 to 40 percent in female rats and 20 to 25 percent for both sexes combined. Results of a single 4-hr exposure to 0.88 mg/l with a CxT value similar to those in the 3.09 and 3.15 mg/l 1-hr studies caused no deaths in either sex, thereby suggesting that exposure concentration, rather

Table 2. EFFECTS OF EXPOSURE TO RP/BR AEROSOL ON FREE CELLS LAVAGED FROM THE LUNGS OF RATS[a]

Exp No.	RP/BR Exposure: Conc, mg/l Mean ± SD[b]	hr/day	No. of daily exp	CXT	Rats: Age wk	Sex	n	Total cells x 10^6	% macrophage	Protein µg/10^5 cells
33	0	4.0	1	0	12	M	4	11.0 ± 1.5	98.3 ± 0.8	19.8 ± 1.0
	0.88 ± 0.02	4.0	1	3.6			8	9.4 ± 2.1	97.6 ± 1.2	23.9 ± 1.7
	0	4.0	1	0	11	F	4	9.8 ± 1.4	96.5 ± 2.5	19.2 ± 1.4
	0.88 ± 0.02	4.0	1	3.6			8	8.0 ± 1.1	96.5 ± 2.8	23.1* ± 1.1
36A	0	1.0	1	0	13	M	9	12.6 ± 1.3	98.8 ± 0.8	20.0 ± 1.1
	2.22 ± 0.00	1.0	1	2.2			9[c]	7.8** ± 0.8	98.8 ± 0.6	23.8** ± 1.0
69[d]	0	1.0	1	0	11	M	6	6.0 ± 1.0	not done	20.9 ± 1.0
	3.18 ± 0.40	1.0	1	3.2			6	2.3** ± 0.7		37.3** ± 4.0
	0	1.0	1	0	11	F	5	3.7 ± 0.4	not done	19.5 ± 2.6
	3.18 ± 0.40	1.0	1	3.2			6	1.3***± 0.3		23.8 ± 2.7
72	0	1.0	4	0	13	M	4	11.6 ± 1.7	98.5 ± 0.2	18.5 ± 1.5
	0.55 ± 0.03	1.0	4	2.2			5	8.5† ± 0.8	98.2 ± 0.1	23.6** ± 0.8
	0	1.0	4	0	13	F	4	7.7 ± 0.9	98.0 ± 0.7	22.3 ± 1.9
	0.55 ± 0.03	1.0	4	2.2			5	5.9 ± 0.7	99.2 ± 0.4	22.3 ± 1.1

[a] All data are expressed as mean ± S.E. for n number of rats.
[b] Determined gravimetrically from multiple filter-collected aerosol samples.
[c] 8 rats were used for protein assay.
[d] In this experiment only lungs were excised before lavage resulting in much lower cell recovery.

Significant difference from control:
† $p < 0.10$
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Table 3. MORTALITY AND MEAN SURVIVAL TIME[a] OF RATS AFTER SINGLE EXPOSURES TO RP/BR AEROSOL

Exp. No.	RP/BR Aerosol Conc., mg/l Mean	± SD[b]	Exposure Duration, hr	Age of Rats, Wks	Mortality Male D/T	%	Female D/T	%	M+F D/T	%	Mean Survival Time[a], Days Male	Female	M+F
50	3.15	0.27	1.2	7	0/5	0	2/5	40	2/10	20	15.0	10.4	12.7
46B	3.09	0.18	1.0	6	2/10	20	3/10	30	5/20	25	12.2	11.0	11.6
36B	2.62	0.08	1.0	12-13	0/9	0	1/9	11	1/18	6	15.0	14.1	14.6
36A	2.22	0.00	1.1	12-13	0/9	0	0/9	0	0/18	0	15.0	15.0	15.0
46A	2.00	0.33	1.0	6	0/10	0	0/10	0	0/20	0	15.0	15.0	15.0
33	0.88	0.02	4.0	12	0/4	0	0/4	0	0/8	0	15.0	15.0	15.0

[a] Mean survival time determined after recording deaths during exposure day and a 14-day post-exposure observation period (total of 15 days).

[b] Means ± SD calculated from multiple daily filter-determined aerosol mass concentration data.

than duration, is the determining factor.

Body weights of the survivors were monitored at various times for 14 days after the single exposures to 2.00, 2.22, 2.62, 3.09 or 3.15 mg/l of the aerosol. Mean body weights are recorded in Table A-1 of the Appendix. Figure 1 shows the mean body weights of male and female rats which survived the exposures to 2.00, 3.09 and 3.15 mg/l as recorded over a 14-day period. There were marked decreases in body weights of both sexes immediately after the single exposure with gradual recovery of the starting weight range after about 9 days and continued increases thereafter. No control animals were included in any of these studies.

Thus although these preliminary mortality experiments were conducted with rats of various ages they demonstrated that LC_{50} studies with single exposures at logarithmically spaced concentrations, as required in the contract, were not feasible within our system because of the nature of the biologic responses (20 to 25 percent mortality) in the range of aerosol concentration levels physically attainable with the generators (maximum capacity at our standardized 500 l/min airflow rates approximately 3 mg/l for 1 hr).

2. Multiple Exposures

A series of multiple exposures were therefore conducted in which 6- to 8-week-old male and female rats inhaled RP/BR aerosol for 1 hr per day on 5 consecutive days at concentrations ranging from 1.56 to 3.05 mg/l. In another study rats received five daily 4-hr exposures to 0.35 and 0.99 mg/l of RP/BR aerosol. The results from these experiments are shown in Table 4. With four of the experiments (Nos. 65A and B, and 57A and B) control groups exposed to filtered air were also included. Mortality rates, mean survival times, body weights and overall clinical observations were made for a total of 19 days (during the 5 exposure days and for an additional 14-day observation period. Necropsies were done on all animals that died spontaneously during the study. Survivors were necropsied on Day 19.

Mortality and mean survival data show that for the 1-hr exposures, mortality increased from 0 and 10 percent 1.56 mg/l to 80 and 100 percent at 3.05 mg/l for male and female rats resepctively. The overall change in mortality of the two sexes combined was 5 to 90 percent. Mean survival times were correspondingly decreased or unchanged. In the 4-hr exposures, only one female rat died in the entire study and that was at the lower exposure concentration. Observations from the follow-up gross necropsy did not explain the unexpected death of this animal.

The LC_{50} value calculated from the mortality data of the 5 daily 1-hr exposure dose response studies shown in Table 4 for the

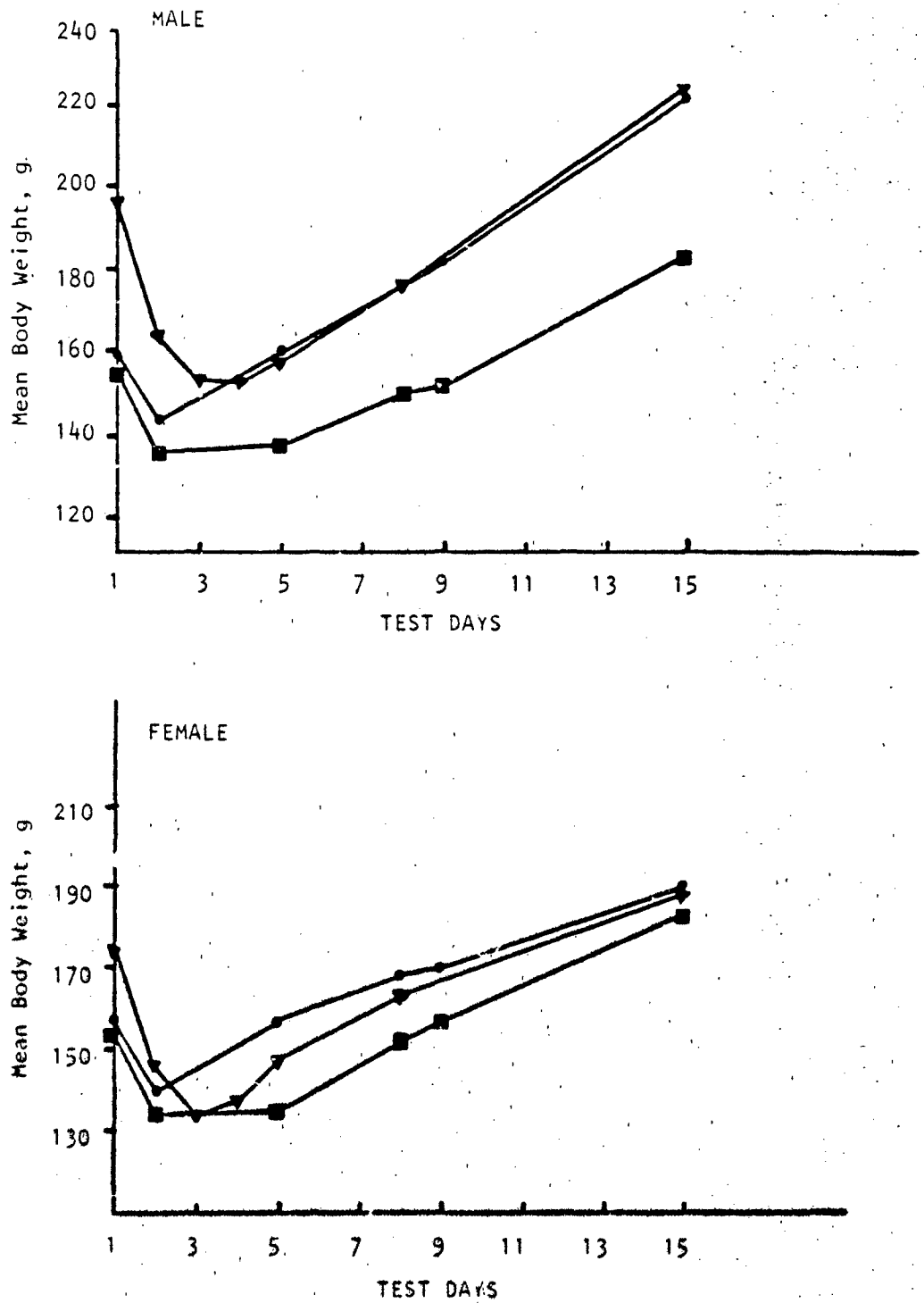

FIGURE 1: MEAN BODY WEIGHTS OF MALE AND FEMALE RATS BEFORE AND AFTER A SINGLE 1-HR EXPOSURE ON DAY 1 TO RP/BR AEROSOLS 2.0 mg/l (●-●), 3.09 mg/l (■-■), or 3.15 mg/l (▼-▼).

Table 4. MORTALITY AND MEAN SURVIVAL TIME[a] OF 6- TO 8-WEEK-OLD MALE AND FEMALE RATS AFTER 5 DAILY EXPOSURES TO RP/BR AEROSOL

Exp. No.	RP/BR Aerosol Exposure Conc., mg/l Mean	± SD[b]	Daily Period, hr	Mortality Male D/T	%	Female D/T	%	M+F D/T	%	Mean Survival Time, Days Male	Female	M+F
52A	3.05	0.29	1.0	9/5	90	5/5	100	9/10	90	6.6	2.0	4.3
65B	2.49	0.07	1.0	4/10	40	5/10	50	9/20	45	12.6	11.1	11.8
52B	1.99	0.13	1.0	3/10	30	4/10	40	7/20	35	13.9	12.6	13.3
65A	1.56	0.10	1.0	0/10	0	1/10	10	1/20	5	19.0	17.8	18.4
65	0		1.0	0/10	0	0/10	0	0/20	0	19.0	19.0	19.0
57B	0.99	0.07	4.0	0/10	0	0/10	0	0/20	0	19.0	19.0	19.0
57A	0.35	0.05	4.0	0/10	0	1/10	10	1/20	5	19.0	17.7	18.4
57	0		4.0	0/10	0	0/10	0	0/20	0	19.0	19.0	19.0

[a] Mean survival time determined after recording deaths during 5 exposure days and a 14-day post-exposure observation period (total of 19 days).

[b] Overall means ±SD calculated from 5 daily means obtained from multiple daily filter-determined aerosol mass concentration determinations.

aerosol concentrations of 0, 1.56, 1.99, 2.49 and 3.05 mg/l was 2.32 mg/l with 95 percent confidence intervals of 1.99 and 2.73 mg/l respectively.

Figures 2 and 3 show the mean body weights in the survivors from these 5 daily 1-hr exposure studies. (The data are recorded in Table A-2 of the Appendix.) It can be seen that at all four concentrations there are marked decreases in body weights of both male and female rats during the exposure period. Although no controls were included with the exposures at 3.05 and 1.99 mg/l (Figure 2) it appears that survivors of the 3.05 mg/l exposure concentration did not even recover their starting weight during the 14-day observation period. The second set of experiments (Figure 3) show definite weight losses after 1.56 and 2.49 mg/l exposures relative to air-exposed conrols with a more marked effect after the exposure to the higher concentration. Although the rate of weight gain appeared to be similar in exposed and control rats after the initial weight loss in the RP/BR exposure groups, the exposed animals never attained the weight of the controls.

The corresponding mean body weights for the 4-hr exposure studies are shown in Figure 4 and recorded in Table A-2 of the Appendix. It appears that for both concentrations there was only a slight initial weight loss in exposed female rats relative to controls and recovery was complete by the end of the observation period. In males there appeared to be a somewhat more marked effect during and after exposure to 0.99 mg/l of the aerosol. The single "dipping" point observed on Day 8 in the 0.35 mg/l exposure group was the result of limited food availability over the week-end because of a non-aligned feeder.

Clinical observations were made twice daily in all six studies during the five exposure days and in the 14-day observation period that followed. Control animals appeared normal throughout. An occasional incidence of crusted eye was observed in 5 to 10 percent of all groups including the controls. As the RP/BR concentration was increased in the four 1-hr exposure experiments from 1.56 to 3.05 mg/l the fraction of animals showing labored breathing at some time during the observation period increased from 20 percent (occasional incidence) to 100 percent (through the entire 19 days). The fraction of animals which were lethargic at some time increased from 20 percent to 60 percent. Reddish discharge from the nose was observed in 30 percent of the animals exposed to 3.05 mg/l of the aerosol only.

In the experiments in which the rats were exposed five times for 4 hr to filtered air, 0.35 mg/l RP/BR or to 0.99 mg/l RP/BR, a crusted eye was occasionally seen in 5 to 10 percent of the animals. Ten percent of the controls and 20 percent of the 0.35 mg/l group were hyperreactive at some time. Ten percent of the

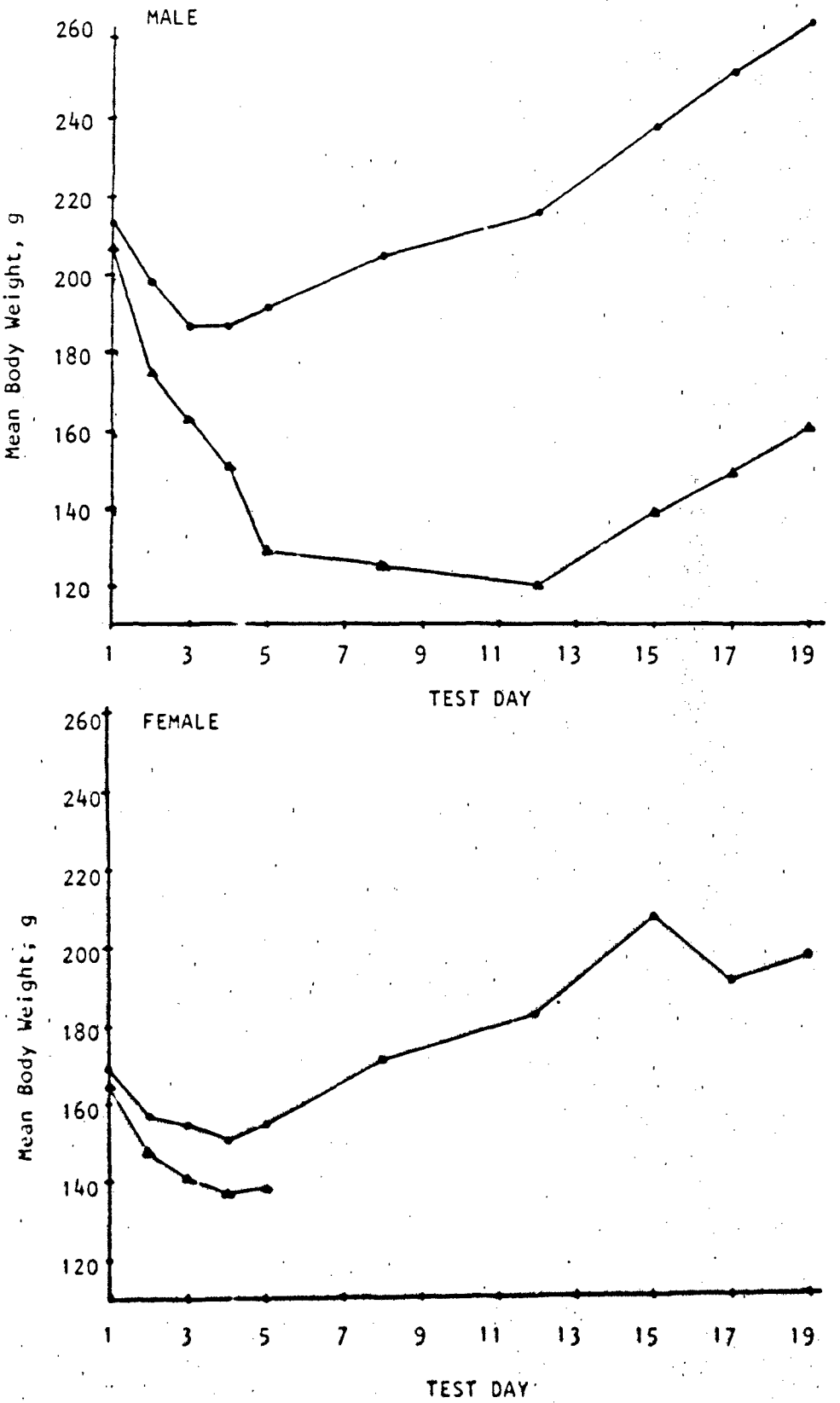

FIGURE 2: MEAN BODY WEIGHT CHANGES IN MALE AND FEMALE RATS DURING AND AFTER 5 DAILY 1-HR EXPOSURES TO RP/BR AEROSOLS 1.99 mg/l (●-●), OR 3.05 mg/l (▲-▲).

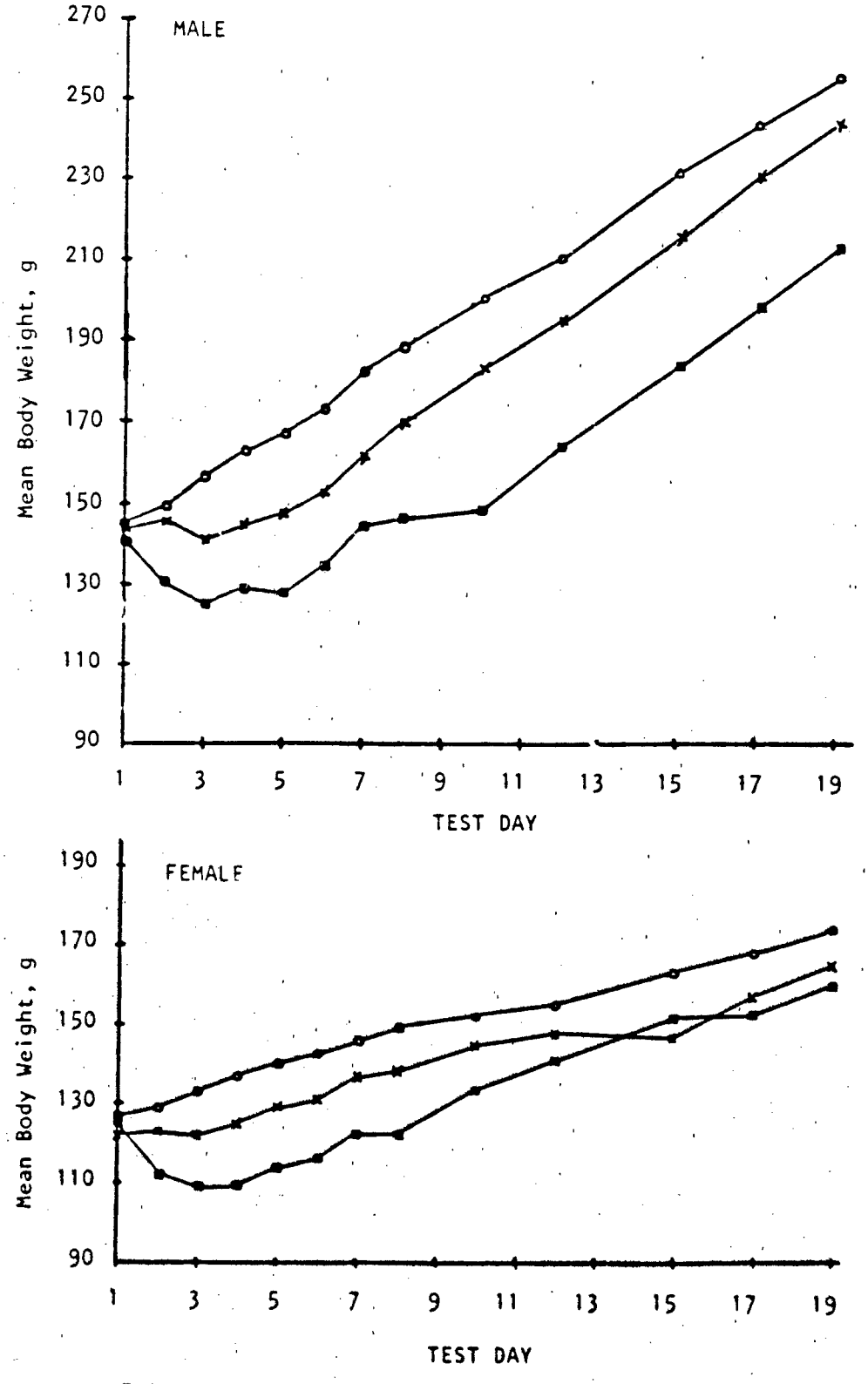

FIGURE 3: MEAN BODY WEIGHT CHANGES IN MALE AND FEMALE RATS DURING AND AFTER 5 DAILY 1-HR, EXPOSURES TO RP/BR AEROSOLS 1.56 mg/l (x-x), OR 2.49 mg/l (■-■), OR TO FILTERED AIR (0-0).

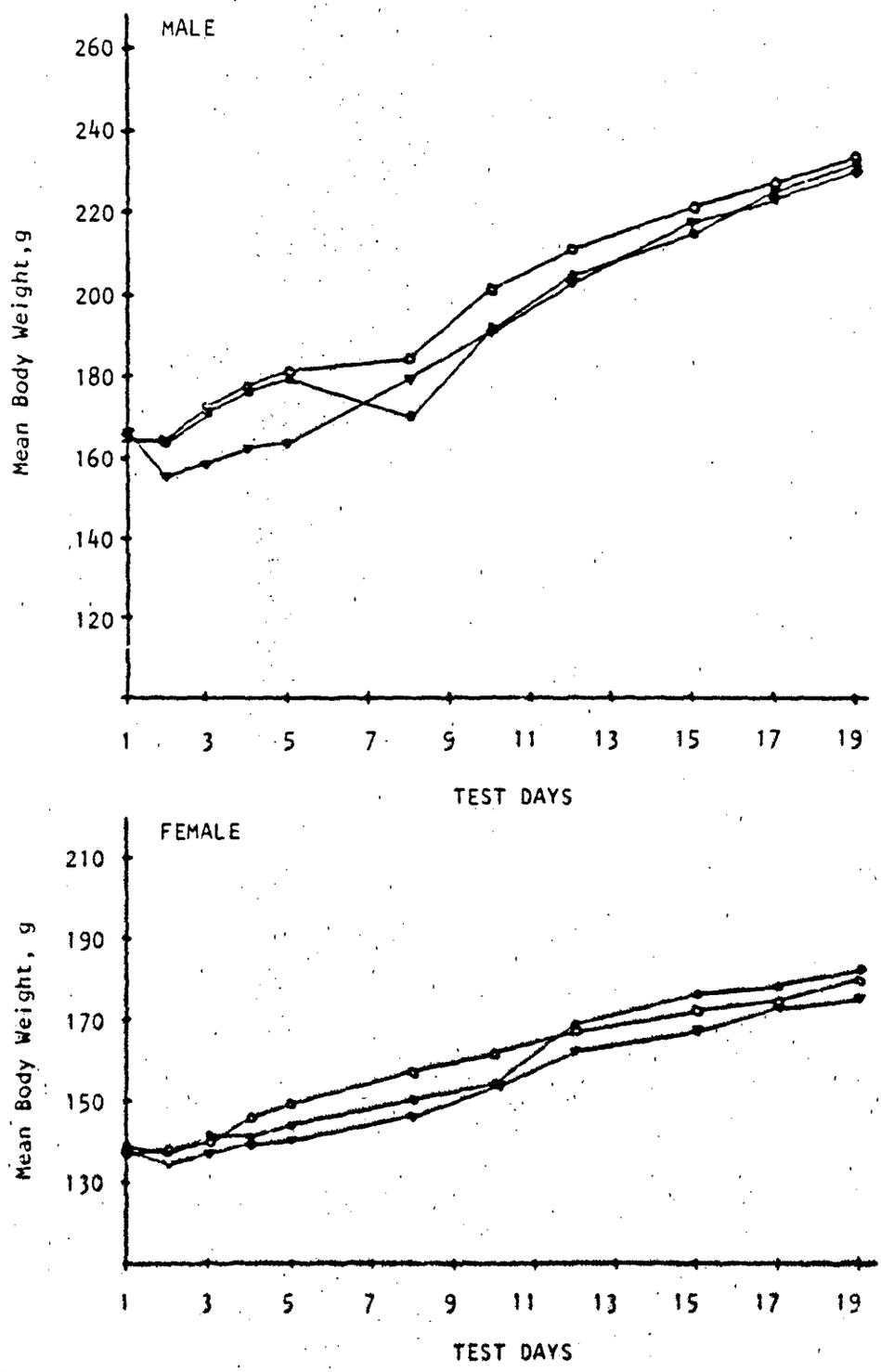

FIGURE 4: MEAN BODY WEIGHT CHANGES IN MALE AND FEMALE RATS DURING AND AFTER 5 DAILY 4-HR EXPOSURES TO RP/BR AEROSOLS 0.99 mg/l (▼-▼), or 0.35 mg/l (●-●), OR TO FILTERED AIR (O - O).

animals exposed to 0.99 mg/l were lethargic at some time during observation.

All animals which died were necropsied and observations were made of lungs, trachea, esophagus, liver, stomach, intestines, cecum. No control animals were included for experiments 52A and 52B (3.05 mg/l and 1.99 mg/l aerosol for five 1-hr exposures). In general, the observations on these spontaneous deaths included overall mottled red lungs with occasional dark red foci, occasional tan foci on the liver, and gas-filled stomach, intestines and cecum. Controls included in other studies and all surviving animals were killed by CO_2 inhalation and exsanguination at the conclusion of the observation period. Control animals (Experiments 57 and 65) exhibited the same mottled red lungs with occasional pinpoint or larger red foci and an occasional tan focus on the liver as observed in the exposed groups (0.35 mg/l and 0.99 mg/l RP/BR for five 4-hr exposures for Experiments 57A and 57B respectively). Observations in necropsies of animals exposed to 1.56 mg/l RP/BR showed a greater incidence of red mottling and red foci in the lungs. Necropsies of animals exposed to 2.49 mg/l RP/BR (Experiment 65B) yielded similar observations with the addition of occasional necrotic foci on the liver in spontaneous deaths.

This comparison of the 1-hr and 4-hr studies in terms of body weight changes and of clinical observations demonstrated marked toxic effects on both parameters after the 1-hr exposures but negligible effects after 4-hr exposures to lower concentrations of the RP/BR. These results again indicate the importance of the exposure concentration relative to duration since for similar or higher CxT values in the 4-hr studies there were minimal effects compared to the 1-hr exposure.

C. Definitive Studies

These preliminary range finding data were used as a basis for the final design for the Phase II definitive studies. These studies were conducted to provide information on selected biologic response parameters after four daily exposures to 0.5 mg/l of RP/BR aerosol and comparing the effects at 1.0- and 3.5-hr exposure durations. The biologic endpoints selected for this study were pulmonary free cell parameters (total and differential cell counts and cellular protein and ATP levels in the free cells obtained by tracheobronchial lavage), body weights and potential structural changes (gross necropsy and histopathology). Pulmonary bactericidal activity was not chosen for this study because of the very high sensitivity of this assay. The selected parameters were tested immediately following the last exposure and after a 14-day recovery period. In an additional study rats were exposed to 0.5 mg/l of RP/BR for 3.5 hr daily 4 days/week for 4 weeks and examined for histopathologic changes with and without recovery.

1. Experimental Design

The experimental design with exposure group allocations for study Nos. 76, 77 and 78 is shown in Table 5. In Study Nos. 76 and 77 four groups of 14 male and 14 female rats were exposed for 1.0 or 3.5 hr daily, four times per week for one week to 0.5 mg/l of RP/BR aerosol. Four groups were exposed to filtered air. In Study Nos. 76A and B, two RP/BR and two air-treatment groups (I and II: V and VI) were exposed for 1.0 hr per day on 4 consecutive days. In Study Nos. 77A and B, the remaining four treatment groups (III and IV; VII and VIII) received 4 daily 3.5 hr exposures to RP/BR or air. One RP/BR and one air group from each exposure duration (treatment groups I to IV) were sacrificed subsequent to the last exposure. Pulmonary lavages with total and differential cell counts, cellular protein and ATP levels were done for 10 male and 10 female rats from each group. The remaining 4 males and 4 females were killed, necropsied and tissues from 2 rats of each sex were processed for histopathologic examination of the respiratory tract. The other RP/BR and air treatment groups from each exposure duration were maintained for a 14-day recovery period (treatment groups V to VIII) at the end of which the same numbers of animals from each group as listed above were used for pulmonary lavage, necropsy and histopathology.

Study No. 78 was conducted to determine if any gross structural changes in the respiratory tract could be discovered relative to controls from inhalation of 0.5 mg/l of RP/BR aerosol, 3.5 hr daily, four days per week over a four-week period at the completion of the exposures and after a 14-day recovery period. Pulmonary lavage parameters were not examined in this study.

Animals in all three studies were weighed on a regular schedule and clinical observations for toxic signs were made twice daily except for weekends and holidays.

2. Exposure to RP/BR Aerosol

The rats were exposed to RP/BR aerosol or filtered air in 1-m^3-sized inhalation chambers as described in the methods section. For animals receiving the same exposure regimen but different post-exposure treatments (that is, with or without recovery; such as treatment groups II and VI and IV and VIII, respectively), common chambers were used and, therefore, common exposure concentrations were calculated. However, in order to facilitate the time-consuming and labor-intensive processes of pulmonary lavage and necropsy of the test animals, the exposures were staggered. Exposure of 5 male and 5 female rats from each group for lavage and 2 of each sex for necropsy were started one day and the remaining (5+2) rats per sex per group were started on the next day (Monday through Thursday and Tuesday through Friday, respectively). Therefore, there were actually five days of

Table 5. EXPOSURE GROUP ALLOCATION FOR STUDY NOS. 76, 77 AND 78

Study No.	Treatment Group	Exposure				Recovery Period (14 Days)	No. of Rats per Sex		
		Target Conc. (mg/l)	Hr/day	No/Week	Total Weeks		Total	Lavage	Necropsy
76A	I	0	1.0	4	1	-	14	10	4
	II	0.5	1.0	4	1	-	14	10	4
77A	III	0	3.5	4	1	-	14	10	4
	IV	0.5	3.5	4	1	-	14	10	4
76B	V	0	1.0	4	1	+	14	10	4
	VI	0.5	1.0	4	1	+	14	10	4
77B	VII	0	3.5	4	1	+	14	10	4
	VIII	0.5	3.5	4	1	+	14	10	4
78		0	3.5	4	4	+	20	-	4[a]
	b	0.5	3.5	4	4	+	20	-	4[a]

[a] two rats per sex and per treatment group, were necropsied after the exposures and after the 14-day recovery period, respectively.

[b] no treatment group designation given.

multiple daily chamber readings from which the means and standard deviations (SD) for aerosol mass concentration and particle size values for the entire four exposure day study were calculated. Thus, in Study Nos. 76 and 77, for the 1.0-and 3.5-hr aerosol exposures, 15 (3 per day on 5 days) and 20 (4 per day on 5 days) filter and integrated photosensor samples, respectively, were collected. For Study No. 78, 64 filter and photosensor samples (4 per day on 16 days) were taken. Aerosol mass concentration and particle size data for the various exposure periods were calculated as overall means from the multiple daily means averaged over the entire exposure period. Percent phosphoric acid was determined from one filter-collected sample per week. The aerosol mass concentration and particle size data for Study Nos. 76, 77 and 78 are summarized in Table 6.

The mass median aerodynamic diameters (MMAD) shown in the table for Study Nos. 76 and 77 were higher than the values we usually observed (0.45 to 0.63 μm). In reviewing the original Quartz Crystal Microbalance impactor data for these studies it was found that for all replicate samples impactor stage No. 7 (cut diameter = 0.84 μm) collected between 45% and 60% of the total particle mass, while stage No. 6 (cut diameter = 1.69 μm) collected nothing. Thus, the resulting aerosol size distribution was one tailed, and, because the mass median aerodynamic diameter is a single point property of the distribution, the median particle size appears markedly different from the expected value. The mass mean aerodynamic diameter, being a property of the overall distribution, was not as severely affected with values (0.61, 0.57 and 0.54 for Study Nos. 76, 77 and 78) which were essentially equivalent to mean values previously determined under similar conditions during the aerosol homogenity studies of Phase I.

3. Mortality and Clinical Observations

No deaths occurred in any of the studies. All animals appeared normal during the exposure interval and 14-day recovery period except for 2 males for which crusted eyes were recorded during the recovery period. No other treatment-related changes were seen.

4. Body Weights

According to the experimental design for Study Nos. 76 and 77 only the animals designated for lavage were weighed before each of the four exposures and at predetermined intervals during the recovery period. Mean body weights are summarized for treatment groups I through VIII for the four exposure days in Table A-3 and those for recovery animals (treatment groups V through VIII) taken over an 18-day period in Table A-4 of the Appendix. Two separate analyses were conducted, one on the results of the 4 exposure days and one over the entire 18-day period including 4 exposure and 14

Table 6. AEROSOL MASS CONCENTRATION, PARTICLE SIZE AND PERCENT H_3PO_4 FOR STUDY NOS. 76, 77 and 78

Study No.	Treatment Group	RP/BR Exposure				Aerosol[a]				
						Mass Concentration, mg/l		Particle Size		
		Hr/day	No/week	Total Weeks	Recovery	Filter Sample	Photosensor	MMAD[b]	σg	H_3PO_4, %
76A	II	1.0	4	1	-	0.62 ± 0.06	0.53 ± 0.07	0.94 ± 0.02	2.28 ± 0.08	60.9
76B	VI	1.0	4	1	+					
77A	V	3.5	4	1	-	0.50 ± 0.02	0.46 ± 0.01	0.78 ± 0.00	2.53 ± 0.03	62.7
77B	VIII	3.5	4	1	+					
78	[c]	3.5	4	4	+	0.51 ± 0.04	0.50 ± 0.05	0.61 ± 0.14	1.92 ± 0.21	67.8 ± 2.48

[a] All data given as Mean ± SD from daily means except for percent H_3PO_4 determined from one filter sample per week. For methods see text.

[b] Mass Median Aerodynamic Diameter.

[c] No treatment group designation given.

recovery days. The data were analyzed for between timepoint differences as well as linear trends in weight gain over time.

A multivariate ANOVA of the 4-day exposure period revealed that exposure duration was not a significant factor (there was no treatment by duration interaction) and that the average level of body weights over the four-day period was not significantly affected. However, a significant exposure by sex interaction ($F=5.46$, $df=4/149$, $p<0.0004$) was found. Table 7 displays the daily body weight means averaged over both durations for control and exposed male and female rats. Inspection of the data demonstrated significant ($p<0.05$) differences for individual time points between exposed and control male but not female rats and that the exposure had a significant effect on the linear rate of weight increase in male but not in female rats.

Analysis of the recovery animals over the entire 18-day period revealed that the previously noted sex by exposure interaction for days 1 to 4 was absent. Again, exposure duration was not found to be a significant factor. A significant overall exposure effect was found ($F=8.63$, $df=4/68$, $p<.0001$). Inspection of the average daily body weight means for control and exposed animals (Table 8) revealed that the average level of body weights was significantly decreased over the entire 18-day period in exposed animals ($p<0.008$) and that individual timepoint comparisons were also significant ($p<0.05$). However, the rate of body weight gain was not significantly different between exposed and control animals. This indicates that treatment produces a decrease in body weight that does not return to within the normal control limits in the recovery period.

The mean body weights measured in Study No. 78 in which rats received 16 exposures of 3.5-hr daily duration for 4 days per week are summarized in Table A-5 of the Appendix. The rats were weighed before the first exposure and then weekly on Fridays for the four exposure weeks and two recovery weeks. Statistical analysis of the body weight data was conducted for both between timepoint differences and trends in body weight measurements over time. No significant treatment by sex interaction was found. The average body weights of exposed animals over time were significantly lower than those of controls ($p<0.002$). However, RP/BR exposure did not affect the overall rate of body weight gains relative to those in controls. As shown in Table 9 significantly lower body weights were seen in exposed rats at all timepoints after the initial preexposure weighing on Day 1: Day 5 ($p<0.0002$), Day 12 ($p<0.000008$), Day 19 ($p<0.0002$), Day 26 ($p<0.008$), Day 33 ($p<0.002$), and Day 39 ($p<0.02$). The attenuation of the probability values on Day 33 and Day 39 indicates a recovery effect in which treated animals return to within the 95 percent limits of controls. These body weight measurements are, however, still significantly different ($p<0.02$) from controls.

Table 7. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS DURING FOUR DAYS OF EXPOSURE TO RP/BR AEROSOLS (Study Nos. 76A and 77A)

Treatment Groups	RP/BR Exposure: Conc. (mg/l)[b] Mean ± SD	RP/BR Exposure: Duration (hr/day)	Sex	Mean Body Weights (g), Determined on Study Day: 1[c]	2	3	4
I,III	0	1.0,3.5	M	201.4	208.7	213.7	219.4
II,IV	0.56 ± 0.07	1.0,3.5	M	201.1	204.3*	208.2*	213.1*
I,III	0	1.0,3.5	F	138.9	142.6	144.1	146.8
II,IV	0.56 ± 0.07	1.0,3.5	F	139.1	141.5	142.9	146.6

[a] Calculated for 20 rats per point, averaging over the 1.0 and 3.5-hr exposure duration, because there was no exposure by duration interaction.

[b] Aerosol mass concentration calculated from daily gravimetric filter readings.

[c] Study Day 1 = first exposure day.

* Significantly different from controls ($p < 0.05$).

Table 8. MEAN BODY WEIGHTS OF MALE AND FEMALE RATS DURING RP/BR AEROSOL EXPOSURE AND RECOVERY DAYS (STUDY NOS. 76B and 77B).

Treatment Group	RP/BR Exposure conc.[a] (mg/l) Mean ± SD	RP/BR Exposure Duration hr/day) for 4 days	Sex	Mean Body Weights (g) Determined on Study Day:[b] 1[c]	2[c]	3[c]	4[c]	5	6
V,VII	0	1.0,3.5[d]	M,F[e]	171.5	176.7	180.0	184.6	188.4	193.1
VI,VIII	0.55 ± 0.09	1.0,3.5	M,F	169.5	172.1*	174.3*	178.6*	181.2*	185.5*

Table 8 (Continued). MEAN BODY WEIGHTS OF MALE AND FEMALE RATS DURING RP/BR AEROSOL EXPOSURE AND AND RECOVERY DAYS (STUDY NOS. 76B and 77B)

Treatment Group	RP/BR Exposure conc.[a] (mg/l) Mean ± SD	RP/BR Exposure Duration hr/day for 4 days	Sex	Mean Body Weight (g) Determined on Day:[b] 8	11	14	16	18
V,VII	0	1.0,3.5[d]	M,F[e]	201.6	211.9	223.5	226.5	230.3
VI,VIII	0.55 ± 0.09	1.0,3.5	M,F	195.0*	205.8*	215.5*	218.8*	222.4*

[a] Calculated from daily gravimetric filter readings for both exposure durations combined.
[b] Study Day 1 = first exposure day.
[c] Exposure days.
[d] Data averaged over the 1.0 and 3.5 hr durations because there was no exposure by duration interaction.
[e] Data combined for 20 male and 20 female rats per point because there was no exposure by sex interaction.
* Significantly different from controls ($p < 0.05$).

Table 9. MEAN BODY WEIGHTS[a] OR MALE AND FEMALE RATS DURING FOUR WEEKS OF EXPOSURE FOUR DAYS/WEEK TO RP/BR AEROSOLS AND TWO WEEKS OF RECOVERY

RP/BR Exposure			Mean Body Weights (g), Determined on Study Day:[c]						
Aerosol Conc.[b] (mg/l)	Duration (hr/day)	Sex	1[d]	5[d]	12[d]	19[d]	26[d]	33[e]	39[e]
0	3.5	M,F[f]	168.8	188.4	214.3	232.5	248.5	266.9	280.3
0.51 ± 0.04	3.5	M,F	168.1	180.1***	203.1***	221.8***	239.8**	257.9*	270.9*

[a] Weights are from 20 or 18 rats of each sex per group on Days 1 through 26 and Days 33 through 39, respectively.

[b] Data given as Mean ± SD calculated from daily gravimetric filter readings.

[c] Study Day 1 = first exposure day.

[d] Exposure period.

[e] Recovery period.

[f] Data was averaged across sex because there was no exposure x sex interaction.

Significant difference from control: * $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

5. Pulmonary Free Cell Parameters

Results of the pulmonary lavage experiments are summarized in Table 10 showing total cell counts, percent macrophages determined from differential counts and total protein and ATP levels in the lavaged cells. Statistical analysis of these data revealed no difference between the effects of 1.0- and 3.5- hr exposures (no duration by exposure interaction) over the entire exposure and observation periods. In the macrophages of rats examined immediately after the fourth exposures a significant exposure by sex interaction was found for ug total protein per 10^5 cells ($p<0.001$). For females, averaging over exposure duration RP/BR aerosol inhalation exposure produced an increase in total protein (control = 25.23 and exposed = 28.09 ug protein per 10^5 cells), whereas males exhibited a decrease (control = 25.24 and exposed = 22.99 ug protein per 10^5 cells). In the absence of other treatment by sex interactions the data obtained from the two sexes were combined for all other parameters in Table 11. In addition, in the absence of an exposure by duration interaction the experimental observations were also averaged over the two exposure durations. When examined immediately after the last RP/BR exposure, (treatment groups I through IV) significant overall decreases in ATP content per 10^5 cell ($p<0.002$) and in ATP per ug protein ($p<0.008$) were observed. Statistical analysis of the data obtained after the recovery period (treatment groups V through VII) revealed no significant exposure-related effects; hence recovery from these exposure effects was complete within 14 days.

6. Gross Necropsy and Histopathologic Observations

Gross observations made on the rats during necropsy for the 4-day and the 4-week exposure studies are summarized in the Appendix in "Pathology Report, Part I". After the 4-day exposure study mottled red lungs were found in many of the rats in both the treated and filtered air groups and in both the exposure and recovery groups. The small number of animals per group makes it difficult to determine the significance of this lesion. Other lesions which occurred with some frequency were urinary calculi in males especially in the recovery group, mottled brown kidneys, and distended uteri. These and the remaining lesions observed were regarded as incidental findings and were present in both the control and treated animals.

No compound related gross pathologic lesions were observed in rats from the experimental group sacrificed after the 4-week exposures, nor in rats sacrificed after two weeks of recovery period. All lesions observed at necropsy were considered to be spontaneous in nature and unrelated to inhalation of RP/BR aerosols.

From the 4-day exposure studies two rats per sex per group

Table 10. EFFECTS OF FOUR DAYS OF EXPOSURE TO RP/BR AEROSOLS ON PULMONARY FREE CELLS LAVAGED FROM THE LUNGS OF MALE AND FEMALE RATS[a]

Study No.	Treatment Group	RP/BR Exposure Conc. (mg/l) Mean ±SD	RP/BR Exposure Duration (hr/day)	Cell Counts: Total x 10^6 Males Mean ± SE	Cell Counts: Total x 10^6 Females Mean ± SE	Cell Counts: % Macrophages Males Mean ± SE	Cell Counts: % Macrophages Females Mean ± SE
76A	I[b]	0	1.0	8.79 ± 0.61	6.57 ± 0.41	99.0 ± 0.4	98.8 ± 0.6
	II[b]	0.62±0.06	1.0	9.20 ± 0.81	6.25 ± 0.47	98.9 ± 0.4	98.5 ± 0.3
77A	III[b]	0	3.5	8.21 ± 0.61	7.15 ± 0.48	99.1 ± 0.4	99.0 ± 0.6
	IV[b]	0.50±0.02	3.5	7.51 ± 0.51	6.06 ± 0.75	98.9 ± 0.5	98.9 ± 0.3
76B	V[c]	0	1.0	11.67 ± 1.70	7.88 ± 0.74	97.1 ± 1.0	98.1 ± 0.7
	VI[c]	0.62±0.06	1.0	10.52 ± 1.02	8.47 ± 0.73	98.7 ± 0.6	98.9 ± 0.3
77B	VII[c]	0	3.5	7.86 ± 1.08	10.03 ± 1.15	98.2 ± 0.4	99.1 ± 0.4
	VIII[c]	0.50±0.02	3.5	12.69 ± 1.40	9.84 ± 1.21	97.8 ± 1.0	98.0 ± 0.4

Table 10 (Continued). EFFECTS OF FOUR DAYS OF EXPOSURE TO RP/BR AEROSOLS ON PULMONARY FREE CELLS LAVAGED FROM THE LUNGS OF MALE AND FEMALE RATS[a]

Study No.	Treatment Group	RP/BR Exposure Conc. (mg/l) Mean ±SD	RP/BR Exposure Duration (hr/day)	µg Protein/10^5 Cells Males Mean ± SE	µg Protein/10^5 Cells Females Mean ± SE	ATP fg x 10^8/10^5 Cells Males Mean ± SE	ATP fg x 10^8/10^5 Cells Females Mean ± SE	ATP fg x 10^6/µg Protein Males Mean ± SE	ATP fg x 10^6/µg Protein Females Mean ± SE
76A	I[b]	0	1.0	26.26 ± 2.10	25.48 ± 0.96	0.59 ± 0.09	0.80 ± 0.11	2.42 ± 0.42	3.18 ± 0.40
	II[b]	0.62±0.06	1.0	21.51 ± 1.10	26.65 ± 0.70	0.48 ± 0.07	0.54 ± 0.10	2.27 ± 0.37	2.11 ± 0.40
77A	III[b]	0	3.5	26.14 ± 1.18	24.99 ± 1.06	1.10 ± 0.13	1.11 ± 0.08	4.18 ± 0.46	4.50 ± 0.33
	IV[b]	0.50±0.02	3.5	24.47 ± 0.91	29.53 ± 1.59	0.86 ± 0.07	1.01 ± 0.11	3.52 ± 0.29	3.51 ± 0.38
76B	V[c]	0	1.0	20.91 ± 0.56	22.41 ± 1.45	0.61 ± 0.09	0.77 ± 0.15	3.00 ± 0.44	3.24 ± 0.55
	VI[c]	0.62±0.06	1.0	23.29 ± 1.44	21.46 ± 0.42	0.55 ± 0.13	0.70 ± 0.09	2.50 ± 0.63	3.27 ± 0.41
77B	VII[c]	0	3.5	21.16 ± 2.37	25.25 ± 2.91	0.60 ± 0.13	0.76 ± 0.14	2.28 ± 0.30	3.00 ± 0.56
	VIII[c]	0.50±0.02	3.5	22.62 ± 3.30	21.21 ± 0.80	0.56 ± 0.14	0.59 ± 0.15	3.28 ± 0.64	2.76 ± 0.60

[a] 10 rats per group.

[b] Tested immediately after the last exposure.

[c] Tested after a 14-day recovery period following the last exposure.

Table 11. OVERALL MEAN VALUES[a] FOR ENDPOINT PARAMETERS MEASURED ON PULMONARY FREE CELLS LAVAGED FROM THE LUNGS OF RATS AFTER EXPOSURE TO RP/BR AEROSOLS

Study No.	Treatment Groups	RP/BR Exposure: Conc. (mg/l) Mean ± SD[b]	RP/BR Exposure: Duration (hr/day) for 4 days	Recovery Period (14 day)	Total cells x 10^6	µg Protein / 10^5 cells	ATP fg x10^8 / 10^5 cells	ATP fg x 10^6 / µg protein
76A, 77A	I,III	0	1.0,3.5	-	7.73	c	0.92	3.64
76A, 77A	II,IV	0.55 ± 0.09	1.0,3.5	-	7.25	c	0.73**	2.85***
76B, 77B	V,VII	0	1.0,3.5	+	9.35	22.79	0.65	2.78
76B, 77B	VI,VIII	0.55 ± 0.09	1.0,3.5	+	10.38	21.42	0.61	2.95

[a] Mean values obtained averaging over replication, sex, and exposure duration since no duration by exposure or sex by exposure interactions were found.

[b] Calculated from daily gravimetric filter readings for both durations.

[c] Not reported because of a significant exposure by sex interaction (see text).

Significant difference from control: ** $p < 0.01$
*** $p < 0.001$

were submitted for histologic evaluations of the respiratory tract. Because gross examination of the kidneys from several animals revealed mottling, the following tissues were trimmed and processed to paraffin blocks: trachea, pulmonary lymph nodes, each lung lobe, nasal turbinates, and kidneys. The paraffin blocks were then shipped to Experimental Pathology Laboratories, Inc. where hematoxylin and eosin stained slides were prepared and examined. The results are summarized in "Pathology Report, Part II" and included in the Appendix.

The microscopic changes and a detailed listing of all tissues evaluated are presented in the Histophatology Incidence Tables. All lesions are summarized by sex and treatment group and presented in the Summary Incidence Tables. A correlation of lesions observed at necropsy with the corresponding microscopic observation, where possible, is presented in the Correlation of Gross and Microscopic Findings Tables. The gross observations in these tables were transcribed from the necropsy sheets provided with the paraffin blocks.

A summary of the results of the microscopic examinations indicate that administration of RP/BR at the concentrations and for the duration of exposure used in this study did not produce treatment-related changes in the kidney, trachea, or the nasal turbinates at the levels examined.

Several animals which recovered for 14 days following either a 1.0 - hour or 3.5-hour exposure (for four days) to RP/BR combustion products had focal areas of interstitial reaction with alveolar macrophages which may be treatment related. However, these animals also had moderate to marked lymphoid hyperplasia of pulmonary lymph nodes which suggests an infectious agent such as Sendai virus, Mycoplasma pulmonis or pneumonia virus of mice (PVM) may have produced these lesions. Although inquiries to Harlan/Sprague-Dawley, Inc., supplier of the rats indicated that results of viral serology and bacteriology assays of this rat shipment showed Sendai and Mycoplasma sp to be negative there was a positive PVM titer.

IV. CONCLUSIONS

Exploratory experiments with male and female Sprague-Dawley rats exposed to RP/BR aerosols ranging from 0.5 to 3 mg/l for durations of 1 to 4 hr showed highly significant decreases in pulmonary bactericidal activity to inhaled ^{35}S-K.pneumoniae in exposed relative to control rats after single as well as multiple exposures. The higher exposure concentrations also produced significant decreases in total cell counts in the pulmonary free cells obtained by tracheobronchial lavage from the exposed rats.

The subsequent mortality studies with exposures to RP/BR aerosols ranging from 2.00 to 3.15 mg/l mass concentration demonstrated that the maximum mortality resulting from a single 1-hr exposure to approximately 3 mg/l of the aerosol (maximum generator capacity) was 20 to 25 percent, while 2.62 mg/l resulted in 6 percent deaths. Thus it became evident that LC_{50} studies with single exposures at logarithmically spaced concentrations, were not feasible in the range of aerosol concentration levels physically attainable with the generators at the air flow rates required in our inhalation exposure system.

A single 4-hr exposure to 0.88 mg/l with a CxT value similar to those in the 3.09 and 3.15 mg/l 1-hr studies caused no deaths thereby suggesting that exposure concentration is the determining factor rather than duration.

A follow-up multiple exposure study with rats inhaling RP/BR aerosols for 1-hr daily for 5 consecutive days at concentrations of 1.56, 1.99, 2.49 and 3.05 mg/l showed mortality rates ranging from 5 to 90 percent with an estimated LC_{50} value of 2.32 mg/l. In contrast, when rats received five daily 4-hr exposures to 0.35 or 0.99 mg/l of RP/BR only one died and comparison of body weights and of clinical observation for the 1- and 4-hr studies showed markedly negative effects after the 1-hr and practically negligable ones after the 4-hr exposures. Thus the importance of exposure concentration over duration in terms of producing effects at comparable CxT values became evident again.

In the definitive studies male and female rats received four daily exposures to 0.5 mg/l of RP/BR aerosol for 1.0 or 3.5 hr durations. In an additional experiment rats were exposed to 0.5 mg/l of RP/BR for 3.5 hr daily 4 days per week for 4 weeks.

No deaths occurred in any of the studies and generally no treatment-related clinical observations were seen during any of the exposures on the following 14-day recovery periods. Statistical analysis of body weights showed that exposure duration was not a significant factor. When examined over the exposure and recovery periods combined, the 4-day as well as the 4-week-RP/BR exposures produced significant weight decreases in rats of both sexes and the weights did not return to the normal control levels within the recovery period.

No compound-related gross pathologic lesions were observed during necropsies immediately following the last epxosure, or after a 14-day recovery period in either the 4-day or the 4-week exposure group.

Exposure duration did not have a significant effect on the free cells lavaged from the lungs. Total and differential cell counts were not affected. Cellular ATP levels were significantly

decreased when examined immediately after the fourth exposure, however recovery was complete after 14 days.

Microscopic examination of the tissues immediately following the 4-day exposure and after a 2-week recovery period did not reveal any treatment-related changes in kidneys, trachea and nasal turbinates. The lungs of several animals had focal areas of interstitial reaction with alveolar macrophages which may have been treatment-related. However, in the opinion of the pathologist, the presence of a possible reaction of marked lymphoid hyperplasia of pulmonary lymph nodes which suggests an infectious agent and the information received from the animal supplier about a positive PVM titer in the breeding colony indicates no compound-related effect.

Thus these range finding studies have demonstrated that inhalation of 0.5 mg/l RP/BR aerosol for 1.0 and 3.5 hr daily for 4 days, or for 3.5 hr 4 times weekly for 4 weeks resulted in decreased body weights relative to control animals, but the exposures did not produce gross pathologic changes and the microscopic changes observed in lung tissue could not be considered compound- related. The changes found in pulmonary lavage parameters after four exposures were no longer present after the recovery period. Since the concentration level, 0.5 mg/l, used in these studies produced minimal toxicity, the Phase III studies will use concentration levels at and above this level.

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APPENDIX

TABLES

Table A-1. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS EXPOSED ONE TIME TO RP/BR AEROSOLS AND MONITORED FOR 14 DAYS

Study No.	RP/BR[b] conc. (mg/l)	Sex	Age wks	Mean Body Weights (g) Determined on Study Day: 1[c]	2	3	4	5
36A	2.22±0.00	M	12-13	384.8±15.7(10)	369.6±19.5(10)	367.2± 18.0(10)		
		F	12-13	233.2±11.7(10)	231.2± 6.7(10)	233.4± 6.5(10)		
36B	2.62±0.08	M	12-13	387.5±27.5 (9)	379.0±27.4 (9)	374.9±27.7 (9)		
		F	12-13	221.9±10.6 (9)	220.0±10.9 (9)	220.2±11.8 (9)		
46A	2.00±0.33	M	6	139.0±17.9(10)	123.6±16.5(10)[d]			138.5±23.7(10)
		F	6	137.1± 7.9(10)	119.9± 8.5(10)[d]			135.6± 7.6(10)
46B	3.09±0.18	M	6	133.9±20.7(10)	115.4±11.8(8)[d]			116.6±16.0 (8)
		F	6	133.3± 9.3(10)	114.3± 8.5(9)[d]			115.3±15.4 (7)
50	3.15±0.27	M	7	174.6±15.6(5)	143.4±14.9 (5)	132.2±15.1 (5)	132.4±19.2 (5)	137.4±23.0 (5)
		F	7	154.2±14.0 (5)	126.0±10.5 (4)	114.0± 5.6 (3)	117.3± 9.1 (3)	127.3± 9.0 (3)

Table A-1 (Continued). MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS EXPOSED ONE TIME TO RP/BR AEROSOLS AND MONITORED FOR 14 DAYS

Study No.	RP/BR[b] conc. (mg/l)	Sex	Age wks	Mean Body Weights (g) Determined on Study Day: 7	8	9	15
36A	2.22±0.00	M	12-13	370.3±15.1(10)			382.0±12.9(10)
		F	12-13	233.6± 7.7(10)			226.3±23.9(10)
36B	2.62±0.08	M	12-13	378.8±30.4 (9)			387.2±29.4 (9)
		F	12-13	228.1±10.9 (9)			230.9±10.7 (8)
46A	2.00±0.33	M	6		155.2±28.6(10)	160.8±28.0(10)	196.7±35.4(10)
		F	6		147.7±12.5(10)	149.7±15.4(10)	170.0±11.7(10)
46B	3.09±0.18	M	6		129.0±21.9 (8)	131.1±25.5 (8)	161.8±37.1 (8)
		F	6		131.7±17.0 (7)	137.4±15.9 (7)	163.4±14.7 (7)
50	3.15±0.27	M	7		155.0±24.5 (5)		202.6±34.2 (5)
		F	7		143.0± 6.6 (3)		168.3± 4.9 (3)

[a] Data given as Mean ± SD (number of rats).

[b] Mean ± SD for gravimetric filter samples.

[c] Exposure day = Study Day 1.

[d] These weights were recorded after exposure on Day 1.

Table A-2. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS[b] EXPOSED FIVE TIMES TO RP/BR AEROSOLS AND MONITORED FOR 14 DAYS

Study No.	RP/BR Conc[c] mg/ml Mean ± SD	Sex	Mean Body Weights (g) Determined on Study Day: 1[d,e]	2[e]	3[e]	4[e]	5[e]
52A	3.05±0.29	M	206.6±11.2 (5)	174.9±11.6 (5)	163.0±14.5 (5)	150.5±15.6 (4)	130.0± 9.2 (2)
		F	166.6±18.5 (5)	148.1± 7.7 (4)	140.8± 9.7 (4)	137.0 (1)	138.0 (1)
52B	1.99±0.13	M	213.2±21.1(10)	198.0±17.5(10)	187.1±22.3 (9)	186.8±22.8 (8)	192.0±21.7 (7)
		F	169.2±10.8(10)	156.6±15.0(10)	154.8± 6.9 (8)	151.5±12.5 (8)	155.4±10.6 (7)
57	0	M	205.1±10.5(10)	204.3±10.7(10)	211.8±10.4(10)	217.2±10.9(10)	221.1±11.2(10)
		F	137.4± 6.0(10)	137.8± 6.8(10)	140.2± 4.4(10)	146.3± 6.3(10)	148.5± 6.7(10)
57A	0.35±0.05	M	205.1±11.1(10)	204.0±12.6(10)	210.7±12.7(10)	216.0±12.8(10)	219.3±12.4(10)
		F	138.6± 8.2(10)	136.7± 9.6(10)	140.7±13.5(10)	141.4±15.1(10)	143.8±17.5(10)
57B	0.99±0.07	M	205.8±12.0(10)	194.9±12.3(10)	198.1±10.4(10)	202.1±10.3(10)	203.4± 9.8(10)
		F	138.0± 7.0(10)	135.1± 8.9(10)	137.2± 8.6(10)	139.3±10.6(10)	149.8±10.1(10)
65	0	M	145.4±10.9(10)	149.1±11.5(10)	157.3±12.1(10)	162.8±11.7(10)	167.5±11.3(10)
		F	127.4± 8.4(10)	129.1± 7.6(10)	132.9± 8.2(10)	137.5± 7.3(10)	140.1± 7.1(10)
65A	1.56±0.10	M	144.3±10.7(10)	145.9± 9.3(10)	141.2±13.4(10)	144.9±11.8(10)	147.9±12.3(10)
		F	121.9±12.0(10)	123.3± 8.4(10)	121.6±10.0(10)	125.3±11.1(10)	128.9± 9.0(10)
65B	2.49±0.07	M	140.8±16.0(10)	131.1±16.2(10)	125.5±15.8(10)	128.8±16.8 (8)	128.4±19.0 (8)
		F	124.8±11.8(10)	112.4±11.0(10)	109.1±10.5 (8)	108.9±10.4 (8)	113.8± 9.7 (6)

Table A-2 (Continued). MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS[b] EXPOSED FIVE TIMES TO RP/BR AEROSOLS AND MONITORED FOR 14 DAYS

Study No.	RP/BR Conc[c] mg/ml Mean ± SD	Sex	Mean Body Weights (g) Determined on Study Day: 6	7	8	10
52A	3.05±0.29	M			125.0 (1)	
		F				
52B	1.59±0.13	M			204.9±20.9 (7)	
		F			171.1± 8.0 (7)	
57	0	M			223.7±29.8(10)	240.5±18.4(10)
		F			157.0± 7.6(10)	161.4± 7.2(10)
57A	0.35±0.05	M			209.9±38.3(10)	232.2±22.2(10)
		F			150.0±25.4(10)	153.8±30.3(10)
57B	0.99±0.07	M			219.4± 7.9(10)	231.2± 8.1(10)
		F			145.4±20.4(10)	154.4±13.9(10)
65	0	M	173.3±12.5(10)	181.7±12.9(10)	188.4±13.2(10)	200.5±14.5(10)
		F	142.3± 8.2(10)	146.4± 8.7(10)	149.1± 9.5(10)	152.7± 9.6(10)
65A	1.56±0.10	M	152.6±12.1(10)	162.4±12.7(10)	169.8±11.8(10)	183.7±12.4(10)
		F	131.2± 7.4(10)	136.5± 8.4(10)	137.9± 9.9(10)	146.4± 9.0 (9)
65B	2.49±0.07	M	135.2±19.8 (6)	144.8±21.6 (6)	146.5±29.1 (6)	149.3±32.6 (6)
		F	116.2±10.8 (6)	121.7± 9.8 (6)	122.0±12.6(6)	135.2± 9.2 (5)

Table A-2 (Continued). MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS[b] EXPOSED FIVE TIMES TO RP/BR AEROSOLS AND MONITORED FOR 14 DAYS

Study No.	RP/BR Conc[c] mg/ml Mean ± SD	Sex	Mean Body Weights (g) Determined on Study Day: 12	15	17	19
52A	3.05±0.29	M	120.0 (1)	139.0 (1)	149.0 (1)	161.0 (1)
		F				
52B	1.99±0.13	M	216.3±40.4 (7)	238.0±43.2 (7)	250.7±40.3 (7)	262.9±39.1 (7)
		F	182.3±16.5 (6)	177.7±32.9 (6)	190.2±20.5 (6)	195.7±16.6 (6)
57	0	M	251.3±17.1(10)	260.9±17.7(10)	266.7±17.2(10)	273.0±17.4(10)
		F	166.6± 7.1(10)	171.7± 7.5(10)	173.8± 9.8(10)	178.5±11.0(10)
57A	0.35±0.05	M	244.8±19.6(10)	254.8±15.5(10)	265.4±15.5(10)	271.6±15.6(10)
		F	168.2±10.5 (9)	175.8±12.5 (9)	177.7±14.1 (9)	182.0±12.5 (9)
57B	0.99±0.07	M	244.2± 8.8(10)	256.8± 8.9(10)	263.6±10.3(10)	270.2±10.7(10)
		F	162.3±13.0(10)	167.4±11.8(10)	172.9±12.3(10)	175.3±13.7(10)
65	0	M	211.2±13.7(10)	231.8±16.0(10)	243.7±15.5(10)	255.6±16.8(10)
		F	155.7±10.3(10)	163.8±11.3(10)	168.8±13.4(10)	174.8±11.1(10)
65A	1.56±0.10	M	196.2±12.6(10)	217.0±12.6(10)	230.9±14.3(10)	244.2±15.3(10)
		F	149.4± 9.4 (9)	147.7±19.7 (9)	157.4±12.6 (9)	166.3±12.9 (9)
65B	2.49±0.07	M	165.0±29.0 (6)	185.2±26.2 (6)	198.5±28.8 (6)	211.5±28.9 (6)
		F	141.8± 9.2((5)	152.8± 8.6 (5)	153.6±17.4 (5)	160.8±16.9 (5)

[a] Data given as Mean ± SD (number of rats).

[b] Rats were 6 to 8 weeks old when used.

[c] Mean ± SD for gravimetric filter samples.

[d] Study Day 1 = first exposure day.

[e] Exposure days.

Table A-3. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS DURING FOUR DAILY EXPOSURES TO RP/BR AEROSOL

Treatment Group	RB/BR Aerosol: Aerosol Conc.[b] (mg/l)	RB/BR Aerosol: Duration (Hr/day)	Sex	Mean Body Weights (g), Determined on Study Day: 1[c,d]	2[d]	3[d]	4[d]
I,V	0	1.0	M	202.4±8.1	209.3±10.0	215.0±8.9	219.9±9.3
			F	138.1±8.8	141.9± 7.9	142.4±8.7	145.1±8.3
II,VI	0.62±0.06	1.0	M	201.8±6.8	205.2± 5.6	210.2±6.3	214.4±7.5
			F	137.0±6.9	140.1± 6.7	141.7±7.3	145.0±6.0
III,VII	0	3.5	M	200.3±9.6	208.0± 8.1	212.5±8.6	218.9±8.7
			F	139.7±7.1	143.4± 8.3	145.7±7.9	148.4±8.4
IV,VIII	0.50±0.02	3.5	M	200.4±9.1	203.4± 8.3	206.3±8.5	211.7±9.7
			F	141.3±6.6	142.9± 5.9	144.3±5.3	148.2±6.9

[a] Mean ± SD from body weights of 20 male or female rats, respectively.

[b] Mean ± SD calculated from the mean daily gravimetric filter determinations.

[c] Study Day 1 = first exposure day.

[d] Exposure days.

Table A-4. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS DURING FOUR DAILY EXPOSURES TO RP/BR AEROSOLS AND FOLLOWED BY 14 DAYS OF RECOVERY

Treatment Group	RP/BR Exposure: Aerosol conc.[b] (mg/l)	RP/BR Exposure: Duration (hr/day)	Sex	Mean Body Weights (g) Determined on Study Day: 1[c,d]	2[d]	3[d]	4[d]	5
V	0	1.0	M	202.2± 8.5	207.2±11.4	213.6±10.0	219.0± 9.6	222.4±11.6
			F	137.2± 6.0	141.3± 6.4	140.9± 6.8	143.9± 7.4	148.1± 7.6
VI	0.62±0.06	1.0	M	201.8± 8.9	205.1± 7.0	209.5± 8.3	213.9± 9.6	217.0±10.1
			F	133.5± 5.6	136.3± 3.7	137.1± 5.4	141.0± 5.3	144.2± 5.2
VII	0	3.5	M	204.2± 8.1	211.5± 6.0	216.0± 5.0	222.6± 6.1	226.6± 5.3
			F	139.2± 7.6	143.4±10.2	146.2± 7.9	149.5± 7.1	152.7± 8.0
VIII	0.50±0.02	3.5	M	199.6± 8.7	201.9± 8.1	204.8± 8.3	210.4± 9.9	212.7± 6.3
			F	143.1± 7.5	144.9± 5.4	145.7± 5.0	148.9± 7.4	150.8± 6.7

(Page 1 of 2)

Table A-4 (Continued). MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS DURING FOUR DAILY EXPOSURES TO RP/BR AEROSOLS AND FOLLOWED BY 14 DAYS OF RECOVERY

Treatment Group	RP/BR Exposure: Aerosol conc.[b] (mg/l)	RP/BR Exposure: Duration (hr/day)	Sex	Mean Body Weights (g) Determined on Study Day: 6	8	11	14	16	18
V	0	1.0	M	227.6±10.8	238.5±13.8	252.4±13.9	267.8±17.1	272.0±17.8	276.3±18.5
			F	151.3± 8.3	155.0± 7.1	163.1± 8.8	168.5± 8.5	170.0±10.7	170.5± 8.7
VI	0.62±0.06	1.0	M	223.3±10.7	236.0±11.9	252.7±15.5	264.1±17.2	269.9±17.7	273.7±17.5
			F	147.8± 5.8	152.2± 6.2	160.4± 4.5	171.1± 8.7	173.1± 8.3	175.9± 8.8
VII	0	3.5	M	232.9± 7.1	245.3± 6.9	258.7±13.4	273.0±11.5	278.0±11.0	286.5± 9.3
			F	156.7± 6.5	163.3± 8.5	168.8± 6.6	179.2± 7.9	180.4± 8.2	183.3± 7.7
VIII	0.50±0.02	3.5	M	219.2±10.2	230.2±10.7	243.5±13.8	255.7±12.6	259.3±12.6	265.6±14.7
			F	151.5± 7.4	161.6± 7.5	166.7± 9.5	170.9±17.2	172.9±14.6	174.2±16.7

[a] Mean ± SD from body weights of 10 male or female rats respectively.

[b] Mean ± SD for aerosol mass concentration calculated from daily mean gravimetric filter determinations.

[c] Study Day 1 = first exposure day.

[d] Exposure days.

Table A-5. MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RATS DURING FOUR WEEKS OF EXPOSURE FOUR DAYS/WEEK TO RP/BR AEROSOL AND TWO WEEKS OF RECOVERY

RP/BR Exposure			Mean Body Weights (g), Determined on Study Day:						
Aerosol Conc.[a] (mg/l)	Duration (hr/day)	Sex	1[b,c]	5[c]	12[c]	19[c]	26[c]	33[d]	39[d]
0	3.5	M	199.2 ± 9.8	224.4 ± 10.1	258.3 ± 10.3	281.2 ± 10.7	302.7 ± 12.3	328.2 ± 13.1	345.3 ± 15.6
0.51 ± 0.04	3.5	M	199.3 ± 8.3	214.1 ± 9.1	244.9 ± 11.4	267.1 ± 13.9	289.8 ± 16.7	315.8 ± 20.8	332.9 ± 20.1
0	3.5	F	138.3 ± 8.0	152.3 ± 8.2	170.3 ± 8.6	183.7 ± 10.7	194.3 ± 13.3	205.6 ± 16.3	215.2 ± 18.3
0.51 ± 0.04	3.5	F	136.8 ± 8.2	146.1 ± 8.4	161.3 ± 8.7	176.5 ± 9.3	189.8 ± 10.6	199.9 ± 13.0	208.8 ± 14.2

[a] Data given as Mean ± SD for aerosol mass concentration calculated from daily gravimetric filter readings and for weights from 20 or 18 rats per group on Days 1 through 26 and Days 33 through 39, respectively.

[b] Study Day 1 = first exposure day.

[c] Exposure period.

[d] Recovery period.

PATHOLOGY REPORT
PART I: GROSS OBSERVATIONS (IITRI)

FOUR-DAY INHALATION TOXICITY STUDY OF RP/BR IN RATS:

Pathology and Pulmonary Lavage (Study 76/77)

PATHOLOGY SYNOPSIS

No compound-related gross pathologic lesions were observed in Sprague-Dawley rats from the experimental groups sacrificed after treatment which they received and sacrificed 14 days after their last exposure.

Microscopic examination of the tissues did not reveal any treatment-related changes in kidneys, trachea and nasal turbinates. However, lungs of several animals had focal areas of interstitial reaction with alveolar macrophages which may be treatment-related. It is not clear at this study period whether there was a possible reaction of marked lymphoid hyperplasia of pulmonary lymph nodes which suggests an infectious agent.

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NECROPSY OBSERVATIONS

Introduction

In accordance with the experimental protocol, examination of organs was performed on 64 (32 male and 32 female) Sprague-Dawley rats for IITRI Project L6139, Study Number 76 A and B Phase II. The rats were divided into eight groups according to treatment received, length of exposure, and time of sacrifice.

Thirty-two rats (16 males and 16 females) were exposed to 0.5 mg/l of RP/BR aerosol. Sixteen of the 32 rats (8 males and 8 females) were exposed for one (1) hour. The remaining 16 rats were exposed for 3-1/2 hours. Both groups were exposed to the aerosol once a day for four consecutive days.

The remaining 32 rats (16 males and 16 females) were exposed to filtered air. As with the aerosol, sixteen of the 32 rats (8 males and 8 females) were exposed for one (1) hour, while the remaining 16 were exposed for 3-1/2 hours. Both groups were exposed to the filtered air once a day for four consecutive days.

Eight rats (4 males and 4 females) from each exposure group (0.5 mg/l RP/BR and filtered air) and at each time exposure (1-hr and 3-1/2 hrs) were sacrificed and necropsied within four hours (0 days) after the last exposure (Exposure Group). The remaining 32 rats were sacrificed and necropsied 14 days after their last exposure (Recovery Group). The group number, treatment, number of animals per group per sex, corresponding exposure levels, time of exposure, and time elapsed before sacrifice are outlined below.

EXPOSURE GROUP	TREATMENT	NUMBER MALES	NUMBER FEMALES	EXPOSURE LEVEL (mg/l)	EXPOSURE TIME (hr)*	TEBS**
I	Filtered Air Control	4	4	0.0	1	0 days
II	RP/BR Aerosol	4	4	0.5	1	0 days
III	Filtered Air Control	4	4	0.0	3 1/2	0 days
IV	RP/BR Aerosol	4	4	0.5	3 1/2	0 days
V	Filtered Air Control	4	4	0.0	1	14 days
VI	RP/BR Aerosol	4	4	0.5	1	14 days
VII	Filtered Air Control	4	4	0.0	3 1/2	14 days
VIII	RP/BR Aerosol	4	4	0.5	3 1/2	14 days

* Hours per day for 4 consecutive days
** TEBS - Time Elapsed Before Sacrifice

Materials and Methods

The rats were sacrificed with carbon dioxide either within four hrs (0 days) or 14 days after the last exposure, bled from the dorsal aorta, and necropsied. The organs were examined and fixed in 10% neutral buffered formalin for a period no less than 48 hrs before further processing. The select organs from 32 of the 64 rats necropsied (2 males and 2 females from each of the eight treatment groups) were submitted to the histology laboratory and were processed according to the experimental design.

Results and Discussion

Mottled red lungs were observed in many of the rats in both the treated and filtered air control groups and in both the exposure and recovery groups. The small number of animals per group makes it difficult to determine the significance of this lesion.

Other lesions which occurred with some frequency were urinary calculi in males especially in the recovery group, mottled brown kidneys, and distended uteri. These and the remaining lesions observed were regarded as incidental findings and were present in both the control and treated animals.

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L6139 PHASE II

INHALATION STUDY - RATS
Initial Exposure Group (0 hrs)

ORGAN Lesion	Group	MALES				FEMALES			
		FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours	FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours
NUMBER OF RATS EXAMINED		4	4	4	4	4	4	4	4
NO GROSS LESIONS		1	1	1	1	2	2	1	1
LUNG									
Mottled red			2	2	3	2	1	3	1
Red (diffuse)		1							
Red foci			1						
Depressed areas		1							
Pale/pale red			1						
Mottled									1
THYMUS									
Mottled red									
Red foci		1	1						
KIDNEY									
Pale brown/tan		1							
Mottled brown									
Mass							1		

L6139 PHASE II

INHALATION STUDY - RATS
Initial Exposure Group (0 hrs)

ORGAN Lesion	MALES				FEMALES			
Group	FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours	FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours
URINARY BLADDER								
Contained calculi			1	1				
Distended with yellow fluid								
SEMINAL VESICLES								
Firm			1					
Yellow			1					
UTERUS								
Distended with clear fluid								2
STOMACH								
Contained blue ingesta								
ILEUM (Peyer's patches)								
Enlarged		1					1	
White		1					1	

I.6139 PHASE II

INHALATION STUDY - RATS
Initial Exposure Group (0 hrs)

ORGAN Lesion	Group	MALES: FILTERED AIR CONTROL 1 Hour	MALES: RP/BR 0.5 mg/l 1 Hour	MALES: FILTERED AIR CONTROL 3½ Hours	MALES: RP/BR 0.5 mg/l 3½ Hours	FEMALES: FILTERED AIR CONTROL 1 Hour	FEMALES: RP/BR 0.5 mg/l 1 Hour	FEMALES: FILTERED AIR CONTROL 3½ Hours	FEMALES: RP/BR 0.5 mg/l 3½ Hours
EYES									
Small									

L6139 PHASE II

INHALATION STUDY - RATS
Recovery Group (14 days)

ORGAN Lesion / Group	MALES: FILTERED AIR CONTROL 1 Hour	MALES: RP/BR 0.5 mg/l 1 Hour	MALES: FILTERED AIR CONTROL 3½ Hours	MALES: RP/BP 0.5 mg/l 3½ Hours	FEMALES: FILTERED AIR CONTROL 1 Hour	FEMALES: RP/BR 0.5 mg/l 1 Hour	FEMALES: FILTERED AIR CONTROL 3½ Hours	FEMALES: RP/BR 0.5 mg/l 3½ Hours
NUMBER OF RATS EXAMINED	4	4	4	4	4	4	4	4
NO GROSS LESIONS	0	1	0	0	1	1	0	1
LUNG								
Mottled red	3	1	3	3	2	3	3	1
Red (diffuse)								
Red foci		2	1	1				
Depressed areas								
Pale/pale red								1
Mottled								
THYMUS								
Mottled red		1	1		1			
Red foci								
KIDNEY								
Pale brown/tan								
Mottled brown			2	2		1		
Mass								

L6139 PHASE II

INHALATION STUDY - RATS
Recovery Group (14 days)

ORGAN Lesion	Group	MALES				FEMALES			
		FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours	FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours
URINARY BLADDER									
Contained calculi		3	2	2	2				
Distended with yellow fluid									
SEMINAL VESICLES									
Firm									
Yellow									
UTERUS									
Distended with clear fluid								2	
STOMACH									
Contained blue ingesta						1	1	1	1
ILEUM (Peyer's patches)									
Enlarged									
White									

L6139 PHASE II

INHALATION STUDY - RATS
Recovery Group (14 days)

Organ Lesion	Group	MALES				FEMALES			
		FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/ER 0.5 mg/l 3½ Hours	FILTERED AIR CONTROL 1 Hour	RP/BR 0.5 mg/l 1 Hour	FILTERED AIR CONTROL 3½ Hours	RP/BR 0.5 mg/l 3½ Hours
EYES									
Small						1			

FOUR-WEEK INHALATION TOXICITY STUDY OF RP/BR IN RATS

(Study 78)

NECROPSY OBSERVATIONS

No compound related gross pathologic lesions were observed in Sprague-Dawley rats from the experimental group sacrificed after 16 exposures at 0.5 mg/l for 3.5 hours, nor in rats sacrificed after two weeks recovery period. All lesions observed at necropsy were considered to be spontaneous in nature and unrelated to treatment with Red Phosphorus/Butyl Rubber Combustion Products.

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L6139
SN 78

NECROPSY OBSERVATIONS
Rats Sacrificed After 16 Exposures

ORGANS Lesions	FILTERED AIR CONTROL Males	FILTERED AIR CONTROL Females	3.5 HOUR EXPOSURE 0.5 mg/l BP/BR Males	3.5 HOUR EXPOSURE 0.5 mg/l BP/BR Females
LUNGS				
Scattered red foci	1	-	-	2
Mottled red	-	2	-	2
KIDNEYS				
Mottled red	-	1	2	-
UTERUS				
Distended	-	-	-	-
URINARY BLADDER				
Calculi	2	-	2	-
STOMACH				
Blue ingesta present in the lumen	1	1	-	-

L6139
SN 78

NECROPSY OBSERVATIONS
Recovery Sacrifice

ORGANS Lesions	FILTERED AIR CONTROL Males	FILTERED AIR CONTROL Females	3.5 HOUR EXPOSURE 0.5 mg/l BP/BR Males	3.5 HOUR EXPOSURE 0.5 mg/l BP/BR Females
LUNGS				
Scattered red foci	-	-	-	-
Mottled red	1	1	2	1
KIDNEYS				
Mottled red	-	-	1	-
UTERUS				
Distended	-	1	-	-
URINARY BLADDER				
Calculi	2	-	-	-
STOMACH				
Blue ingesta present in the lumen	1	1	-	-

PATHOLOGY REPORT
PART II: HISTOPATHOLOGY (EPL)

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

IITRI PROJECT NUMBER L06139
PHASE II STUDY 76/77

FOUR-DAY INHALATION TOXICITY STUDY OF RP/BR IN RATS: PATHOLOGY AND PULMONARY LAVAGE

PATHOLOGY REPORT

Submitted to:

IIT Research Institute
Chicago, Illinois 60616

September 9, 1983

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

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PATHOLOGY SUMMARY

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

IITRI PROJECT NUMBER L06139
PHASE II STUDY 76/77

FOUR-DAY INHALATION TOXICITY STUDY OF RP/BR IN RATS: PATHOLOGY AND PULMONARY LAVAGE

PATHOLOGY SUMMARY

Microscopic examinations were performed on selected tissues from male and female Sprague-Dawley rats. The study was designed to determine the toxicity of RP/BR (Red Phosphorus/Butyl Rubber) combustion products administered by multiple inhalation exposure as evaluated by histopathology and pulmonary lavage immediately and after a 14-day recovery period. This report contains the histopathologic findings. The experimental design for this study was as follows:

Exposure Group	Group Name	Conc.	Exposure*	No. Animals Male	Female
I - 1 hr.	Filtered Air Control (0 hour)	0	1 hour	14	14
II - 1 hr.	RP/BR (0 hour)	.5 mg/l	1 hour	14	14
III - 3.5 hr.	Filtered Air Control (0 hour)	0	3.5 hour	14	14
IV - 3.5 hr.	RP/BR (0 hour)	.5 mg/l	3.5 hour	14	14
V - 1 hr.	Filtered Air Control (14 day)	0	1 hour + 14 day recovery	14	14
VI - 1 hr.	RP/BR (14 day)	.5 mg/l	1 hour + 14 day recovery	14	14
VII - 3.5 hr.	Filtered Air Control (14 day)	0	3.5 hour + 14 day recovery	14	14
VIII - 3.5 hr.	RP/BR (14 day)	.5 mg/l	3.5 hour + 14 day recovery	14	14

*All exposures are for four (4) consecutive days.

According to protocol, two rats per sex per group were necropsied and gross and histologic evaluations of the respiratory tract were conducted. Gross examination of the kidneys from several animals revealed mottling. Therefore, the following tissues were trimmed and processed to paraffin blocks: trachea, pulmonary lymph nodes, each lung lobe, nasal turbinates, and kidneys. The paraffin blocks were then shipped to Experimental Pathology Laboratories, Inc. where hematoxylin and eosin stained slides were prepared and examined.

RESULTS

The microscopic changes and a detailed listing of all tissues evaluated are presented in the Histopathology Incidence Tables. All lesions are summarized by sex and treatment group and presented in the Summary Incidence Tables. A correlation of lesions observed at necropsy with the corresponding microscopic observation, where possible, is presented in the Correlation of Gross and Microscopic Findings Tables. The gross observations in these tables were transcribed from the necropsy sheets provided with the paraffin blocks.

Several lesions were found in the lung and pulmonary lymph nodes of a few male and female recovery animals that may be treatment related. No lesions at all were detected in the nasal turbinates and only a few animals had focal areas of epithelial hyperplasia in the trachea.

Changes were found in pulmonary lymph nodes, lung, and kidney in both control and treated animals. Minimal to mild lymphocyte hyperplasia and occasionally hemorrhage were found in the pulmonary lymph nodes of most animals. Moderate to marked lymphocyte hyperplasia was usually seen in association with multifocal accumulations of macrophages, lymphocytes, and interstitial thickening in the lung. These changes were more severe in one Group VI male and one Group VI female and in both Group VIII females, but were also present in Group V and Group VII (control) animals. This pulmonary interstitial reaction could be related to the test substance, but the lesions are also compatible with a subclinical Sendai virus or Mycoplasma pulmonis infection. The marked lymphoid hyperplasia of the pulmonary lymph nodes is indicative of intense antigenic stimulation and makes the latter possibility more likely. Renal changes seen in a few animals consisted of minimal to mild tubular hyperplasia, dilatation, and/or lymphocytic infiltrate. These changes are probably the very earliest lesions of chronic progressive nephropathy, a spontaneous syndrome that commonly occurs in laboratory rats.

One interesting renal finding was a nephroblastoma, a malignant embryonal neoplasm, in rat Number 116 which received four one-hour exposures to .5 mg/l RP/BR and was then necropsied. This large tumor was certainly present before the initiation of the experiment and was probably congenital. It is in no way treatment related.

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

CONCLUSION

The results of this microscopic examination indicate that administeration of RP/BR at the concentrations and for the duration of exposure used in this study did not produce treatment-related changes in the kidney, trachea, or the nasal turbinates at the levels examined.

Several animals which recovered for 14 days following either a 1 hour or 3.5 hour exposure (for four days) to RP/BR combustion products had focal areas of interstitial reaction with alveolar macrophages which may be treatment related. However, these animals also had moderate to marked lymphoid hyperplasia of pulmonary lymph nodes which suggests an infectious agent such as Sendai virus or Mycoplasma pulmonis may have produced these lesions.

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W. O. IVERSON, D.V.M.
Diplomate, ACVP

September 9, 1983

WOI/sfh

SUMMARY INCIDENCE TABLES

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE
REPORT CERTIFICATION

Client Name IIT Research Institute Client Study No. L06139 Phase II 76/77

Study Director Dr. William O. Iverson Pathologist Dr. William O. Iverson

Study Title Four-Day Inhalation Toxicity Study of RP/BR in Rats: Pathology and Pulmonary Lavage

Test Article Red Phosphorus/Butyl Rubber Species Sprague-Dawley Rats

All parts of the pathology phase of this study, including the final report, were reviewed by Experimental Pathology Laboratories Quality Assurance Unit on June 20 - September 9, 1983. All findings were reported to the Study Director and Management.

Janet Milazzo

9/9/83

SUMMARY INCIDENCE TABLE

L06139 Phase II
Study 76/77
4 Day Sacrifice
Male Rats

	Group I	Group II	Group III	Group IV		
TRACHEA (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Focal Hyperplasia						
PULMONARY LYMPH NODES						
(NO. EXAMINED)	(2)	(2)	(2)	(2)		
Lymphoid Hyperplasia	2	1	2	2		
Hemorrhage	1					
Macrophage Hyperplasia		1		1		
Edema						
LUNG (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Hemorrhage	1	1		1		
Atelectasis		2	2	2		
Alveolar Macrophages		1				
Focal Lymphocyte Aggregate			1			
Interstitial Thickening						
NASAL TURBINATE (NO. EXAMINED)	(2)	(2)	(2)	(2)		
KIDNEY (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Tubular Hyperplasia			1			
Tubular Dilatation						
Lymphocytic Infiltrate						
Congestion						

SUMMARY INCIDENCE TABLE

LO6139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Male Rats

	Group V	Group VI	Group VII	Group VIII		
TRACHEA (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Focal Hyperplasia		1				
PULMONARY LYM. NODES						
(NO. EXAMINED)	(2)	(2)	(2)	(2)		
Lymphoid Hyperplasia	2	2	2	2		
Hemorrhage		1	1	1		
Macrophage Hyperplasia		1	2	1		
Edema		1	2	1		
LUNG (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Hemorrhage	2	2	1	2		
Atelectasis	1	1	2	1		
Alveolar Macrophages	1	1				
Focal Lymphocyte Aggregate	1	1				
Interstitial Thickening		1				
NASAL TURBINATE (NO. EXAMINED)	(2)	(2)	(2)	(2)		
KIDNEY (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Tubular Hyperplasia	1	2	2	2		
Tubular Dilatation		1	1			
Lymphocytic Infiltrate		1				
Congestion				1		

SUMMARY INCIDENCE TABLE

L06139 Phase II
Study 76/77
4 Day Sacrifice
Female Rats

	Group I	Group II	Group III	Group IV		
TRACHEA (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Focal Hyperplasia						
PULMONARY LYMPH NODES						
(NO. EXAMINED)	(2)	(2)	(2)	(2)		
Lymphoid Hyperplasia	1	2	2	1		
Hemorrhage	1	1	1			
Macrophage Hyperplasia						
Edema						
Yellow-Green Pigment						
LUNG (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Atelectasis	2	1	1	1		
Alveolar Macrophages						
Focal Lymphocyte Aggregate				1		
Interstitial Thickening						
NASAL TURBINATE (NO. EXAMINED)	(2)	(2)	(2)	(2)		
KIDNEY (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Nephroblastoma		1				
Tubular Hyperplasia						
Lymphocytic Infiltrate						

SUMMARY INCIDENCE TABLE

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Female Rats

	Group V	Group VI	Group VII	Group VIII		
TRACHEA (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Focal Hyperplasia				1		
PULMONARY LYMPH NODES						
(NO. EXAMINED)	(2)	(2)	(2)	(2)		
Lymphoid Hyperplasia	2	2	2	2		
Hemorrhage		1		1		
Macrophage Hyperplasia		2		2		
Edema	1					
Yellow-Green Pigment		1		1		
LUNG (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Atelectasis	1		2			
Alveolar Macrophages		1		2		
Focal Lymphocyte Aggregate		1		2		
Interstitial Thickening		1		2		
NASAL TURBINATE (NO. EXAMINED)	(2)	(2)	(2)	(2)		
KIDNEY (NO. EXAMINED)	(2)	(2)	(2)	(2)		
Nephroblastoma						
Tubular Hyperplasia			1			
Lymphocytic Infiltrate			1	1		

HISTOPATHOLOGY INCIDENCE TABLES

Exposure Group	Group Name	Conc.	Exposure*
I - 1 hr.	Filtered Air Control (0 hour)	0	1 hour
II - 1 hr.	RP/BR (0 hour)	. 5 mg/l	1 hour
III - 3.5 hr.	Filtered Air Control (0 hour)	0	3.5 hour
IV - 3.5 hr.	RP/BR (0 hour)	. 5 mg/l	3.5 hour
V - 1 hr.	Filtered Air Control (14 day)	0	1 hour + 14 day recovery
VI - 1 hr.	RP/BR (14 day)	. 5 mg/l	1 hour + 14 day recovery
VII - 3.5 hr.	Filtered Air Control (14 day)	0	3.5 hour + 14 day recovery
VIII - 3.5 hr.	RP/BR (14 day)	. 5 mg/l	3.5 hour + 14 day recovery

*All exposures are for four (4) consecutive days.

HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
4 Day Sacrifice
Male Rats

	Group I							Group II							Group III							Group IV						
ANIMAL NUMBER	82	98						84	100						86	102						88	104					
TRACHEA	X	X						X	X						X	X						X	X					
Focal Hyperplasia																												
PULMONARY LYMPH NODES																												
Lymphoid Hyperplasia	2	1						1							2	1						2	2					
Hemorrhage		1																										
Macrophage Hyperplasia									2													1						
Edema																												
LUNG	X																											
Hemorrhage		2						1															1					
Atelectasis								2	2						1	1						2	1					
Alveolar Macrophages									1																			
Focal Lymphocyte Aggregate															1													
Interstitial Thickening																												
NASAL TURBINATE	X	X						X	X						X	X						X	X					

Key P = Present N = No Section A = Autolysis X = Not Remarkable

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HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
4 Day Sacrifice
Male Rats

	Group I							Group II							Group III							Group IV						
ANIMAL NUMBER	82	98						84	100						86	102						88	104					
KIDNEY	X	X						X	X							X						X	X					
Tubular Hyperplasia															1													
Tubular Dilatation																												
Lymphocytic Infiltrate																												
Congestion																												
ILEUM								N																				
THYMUS								N																				
URINARY BLADDER																							N					

EPL Experimental Pathology Laboratories, Inc.

Key- P = Present
1 = Minimal
5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Male Rats

	Group V							Group VI							Group VII							Group VIII						
ANIMAL NUMBER	90	106						92	108						94	110						96	112					
TRACHEA	X	X							X						X	X						X	X					
Focal Hyperplasia								2																				
PULMONARY LYMPH NODES																												
Lymphoid Hyperplasia	1	3						1	4						2	1						2	2					
Hemorrhage								1								1						1						
Macrophage Hyperplasia									2						2	1							2					
Edema								1							2	1							1					
LUNG																												
Hemorrhage	1	1						1	1							2						1	1					
Atelectasis		2						2							1	2							1					
Alveolar Macrophages		1							2																			
Focal Lymphocyte Aggregate		1							3																			
Interstitial Thickening									3																			
NASAL TURBINATE	X	X						X	X						X	X						X	X					

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Key P = Present N = No Section A = Autolysis X = Not Remarkable
1 = Minimal 2 = Slight 3 = Moderate 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

LQ6139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Male Rats

	Group V							Group VI							Group VII							Group VIII						
ANIMAL NUMBER	90	106						92	108						94	110						96	112					
KIDNEY		X																										
Tubular Hyperplasia	1							1	1						1	2						1	1					
Tubular Dilatation								1								2												
Lymphocytic Infiltrate								1																				
Congestion																						2						
ILEUM																												
THYMUS								N								N												
URINARY BLADDER	N							N							N	N							N					

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

Exposure Group	Group Name	Conc.	Exposure*
I - 1 hr.	Filtered Air Control (0 hour)	0	1 hour
II - 1 hr.	RP/BR (0 hour)	. 5 mg/l	1 hour
III - 3.5 hr.	Filtered Air Control (0 hour)	0	3.5 hour
IV - 3.5 hr.	RP/BR (0 hour)	. 5 mg/l	3.5 hour
V - 1 hr.	Filtered Air Control (14 day)	0	1 hour + 14 day recovery
VI - 1 hr.	RP/BR (14 day)	. 5 mg/l	1 hour + 14 day recovery
VII - 3.5 hr.	Filtered Air Control (14 day)	0	3.5 hour + 14 day recovery
VIII - 3.5 hr.	RP/BR (14 day)	. 5 mg/l	3.5 hour + 14 day recovery

*All exposures are for four (4) consecutive days.

HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
4 Day Sacrifice
Female Rats

	Group I							Group II							Group III							Group IV						
ANIMAL NUMBER	1 1 4	1 3 0						1 1 6	1 3 2						1 1 8	1 3 4						1 2 0	1 3 6					
TRACHEA	X	X						X	X						X	X						X	X					
Focal Hyperplasia																												
PULMONARY LYMPH NODES																						X						
Lymphoid Hyperplasia		1						1	2						1	2							3					
Hemorrhage	1							1							1													
Macrophage Hyperplasia																												
Edema																												
Yellow-Green Pigment																												
LUNG								X							X													
Atelectasis	2	2							2							1						1						
Alveolar Macrophages																												
Focal Lymphocyte Aggregate																							1					
Interstitial Thickening																												
NASAL TURBINATE	X	X						X	X						X	X						X	X					

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Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

HISTOPATHOLOGY INCIDENCE TABLE

LO6139 Phase II
Study 76/77
4 Day Sacrifice
Female Rats

	Group I		Group II		Group III		Group IV	
ANIMAL NUMBER	11140	11310	11162	11320	11184	11340	12006	12036
KIDNEY	X	X		X	X	X	X	X
Nephroblastoma			P					
Tubular Hyperplasia								
Lymphocytic Infiltrate								
UTERUS								N
THYMUS								
STOMACH								

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

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HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Female Rats

ANIMAL NUMBER	Group V						Group VI						Group VII						Group VIII					
	1 2 2	1 3 8					1 2 4	1 4 0					1 2 6	1 4 2					1 2 8	1 4 4				
TRACHEA	X	X					X	X					X	X					X					
Focal Hyperplasia																				2				
PULMONARY LYMPH NODES																								
Lymphoid Hyperplasia	2	2					2	3					1	2					4					
Hemorrhage								1												1				
Macrophage Hyperplasia							1	2											1	1				
Edema	1																							
Yellow-Green Pigment								1												1				
LUNG	X						X																	
Atelectasis		2											1	1										
Alveolar Macrophages								1											2	2				
Focal Lymphocyte Aggregate								1											3	2				
Interstitial Thickening								1											3	3				
NASAL TURBINATE	X	X					X	X					X	X					X	X				

EPL Experimental Pathology Laboratories, Inc.

Key: P = Present N = No Section A = Autolysis X = Not Remarkable
1 = Minimal 2 = Slight 3 = Moderate 4 = Moderately Severe/High
5 = Severe/High I = Incomplete Section

HISTOPATHOLOGY INCIDENCE TABLE

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice
Female Rats

	Group V							Group VI							Group VII							Group VIII						
ANIMAL NUMBER	122	138						124	140						122	142						128	144					
KIDNEY	X	X						X	X													X						
Nephroblastoma																												
Tubular Hyperplasia																1												
Lymphocytic Infiltrate															1								1					
UTERUS																												
THYMUS		N																										
STOMACH																N												

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Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS TABLES

LQ6139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: I - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
98	Lungs	Red	Hemorrhage

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L06139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: II - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
84	Lungs	Pale red	No corresponding lesion
	Lungs - all lobes	Scattered 0.1 x 0.1 cm dark red foci	Hemorrhage
	Ileum	Peyer's patch, 0.5 x 0.5 x 0.3 cm, white	No section
	Thymus	Scattered dark red foci	No section
100	Lungs	Mottled red	No corresponding lesion

LO6139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: III - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
86	Lungs	Mottled red	No corresponding lesion

06139 Pha_e II
tudy 76/77
Day Sacrifice

CORRELATION OF GROSS AND M SCOPIC FINDINGS

pecies: Rats Sex: Males Group Number: IV - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
104	Lungs	Mottled red	Hemorrhage
	Urinary bladder	Proteinaceous calculus present in lumen	No section

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: V - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
90	Lungs - all lobes	Red mottled	Hemorrhage
	Urinary bladder	White calculus	No section
106	Lungs	Mottled red	Hemorrhage

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: VI -- Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
92	Lungs	Scattered dark red foci	Hemorrhage
	Thymus	Mottled red	No section
	Urinary bladder	White calculus	No section
108	Lungs	Mottled red	Focal lymphocyte aggregate

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: VII - Sacrificed Dosage Level 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
94	Lungs	Scattered dark red foci	No corresponding lesion
	Urinary bladder	White calculus	No section
110	Thymus	Mottled red	No section
	Lungs	Mottled red	Hemorrhage
	Kidneys	Mottled brown	No corresponding lesion
	Urinary bladder	White calculus	No section

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Males Group Number: VIII - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
96	Lungs	Mottled red	Hemorrhage
112	Lungs	Mottled red	Hemorrhage
	Kidneys	Mottled brown	No corresponding lesion
	Urinary bladder	White calculus	No section

L06139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: I - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
114	Lungs	Mottled red	No corresponding lesion
130	Lungs	Mottled red	No corresponding lesion

L06139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: II - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
116	Kidney - II, anterior pole	Tan, firm mass, 1.0 x 1.1 x 0.8 cm	Nephroblastoma

L06139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: III - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
134	Lungs	Mottled red	No corresponding lesion

L06139 Phase II
Study 76/77
4 Day Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: IV - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
120	Lungs	Mottled	No corresponding lesion
136	Uterus - horns	Dilated, contain clear fluid	No section

107

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: V - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
138	Thymus	Mottled red	No section

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: VI - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
124	Lungs	Mottled red	No corresponding lesion
140	Lungs	Mottled red	No corresponding lesion
	Kidneys	Mottled brown	No corresponding lesion

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: VII - Sacrificed Dosage Level: 0 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
126	Lungs	Mottled red	No corresponding lesion
142	Stomach	Contains small amount of blue discolored ingesta	No section
	Lungs	Mottled red	No corresponding lesion

110

L06139 Phase II
Study 76/77
14 Day Recovery Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rats Sex: Females Group Number: VIII - Sacrificed Dosage Level: .5 mg/l

Animal Number	Client's Tissue Identification	Client's Gross Observations	Microscopic Observations
128	Lungs	Mottled red	Focal lymphocyte aggregate
144	Lungs	Pale	No corresponding lesion

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